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Mycobacterium

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INTRODUCTION

Up until about 10 years ago, our concern with mycobacteria was occasional and related to identifying organisms from clinical materials. Then, when pondering the *species forma, Mycobacterium leprae* (95), we found a need to know more about *Mycobacterium*. An inspection of Tables 1 and 2 will remind the reader that 50% of all mycobacterial species were not recognized until after 1950, despite the fact that *M. tuberculosis* had been known since 1882. Although these "new" species of mycobacteria had been around since prehistoric times, their acceptance by mycobacteriologists, especially medical mycobacteriologists, was slow due to beliefs and prejudices that held sway following the establishment of *M. tuberculosis* as *the* cause of *tuberculosis*. Expressions such as "atypical mycobacteria" and beliefs such as "chromogenic mycobacteria do not cause pulmonary disease" gained currency during the period 1900 to 1920 when the approach to clinical material was often "to rule in or out" *M. tuberculosis*. Now that the infective potential of many mycobacteria is appreciated (see Table 4) and their identification is easily accomplished (see Table 3), there is a need to go beyond mere identification of mycobacteria and assemble data concerning the *biology* of these versatile microorganisms. The present review is aimed at defining *Mycobacterium* from two general standpoints: (i) their *biology*, including their biosynthetic capacities, the shapes they assume, the basis for their own peculiar acid-fastness, etc. (original work of ours reported herein deals with the *biology* of mycobacteria) and (ii) the reactions they and/or their various products elicit in animal hosts. The immune response of animals to mycobacteria provides us with a further means of characterizing the organisms and their products. Our coverage of immune responses to mycobacteria is somewhat diffuse. To a degree, this reflects the current proliferation of reports attempting to define the components of the immune system and their interaction.

Mammalian immune mechanisms comprise multicomponent systems whose primary selective advantage may have come from their capacity to successfully control growth and differentiation. One aspect of this control may involve surveillance (1152) by adaptive (206) and nonadaptive immune killing (469a) of elements (endogenous or exogenous) foreign to the host. When foreign antigens stimulate an immune system with seemingly exquisite specificity, they do so as alien agents carrying non-*self* markers (*self* includes major histocompatibility antigens [MHC; 77a], ABO blood group antigens, and any as yet to be discovered surface antigens of host phenotypes) toward which the host animal is equipped to respond. When adjuvant-active molecules enhance or depress a host response, they are exhibiting a capacity to regulate an activity of the host. Perhaps, in some cases, their capacity to function as regulators stems from their likeness to regulator molecules of the host. This likeness seemingly would require a neatness of fit such as that which exists between enzymes and their substrates. The accident of such fits no doubt involves the kind of accidents of similarity that account for serological cross-reactions. An interesting example of a bacterial product serving as a regulator molecule in a mammalian immune system is *N*-acetylmuramyl-L-alanyl-D-isoglutamine which, when administered to animals, turns on both antibody synthesis and the development of delayed hypersensitivity to an otherwise "poor" antigen. It has been established that, whereas *N*-acetylmuramic acid can be replaced by *N*-glycolyl-muramic acid in this sugar dipeptide, the γ-glutamyl function of isoglutamine is essential for the adjuvant action of the compound. Does the sugar dipeptide, synthetic or derived from bacterial cell walls (of a wide range of species of bacteria), do its job because it mimics a regulator molecule of the immune system?

The mammalian immune system does not always reject that which is foreign. For example, there is accommodation to foreign-ness in

TABLE 1. *Chronology of our comprehension of the genus Mycobacterium, 1870-1974*[a]

1970	*M. szulgai* (776, see also 1001)[b]
	M. farcinogenes (229; 228, see also [699, 440])
	M. africanum (225; 226)
	M. gastri (1225), *M. triviale* (561; 671)
	M. diernhoferi (157), *M. nonchromogenicum* (1162), *M. simiae* (1241; 582)
	M. vaccae (156)
	M. flavescens (151), *M. gordonae* (151), *M. peregrinum* (151)
1960	
	M. xenopi (1037)
	M. paraffinicum (292)
	M. kansasii (198; 500)
	M. scrofulaceum (940; 941)
1950	*M. terrae* (978; 1225)[c]
	M. intracellulare (275; 996)[d]
	M. ulcerans (746; 370)
1940	
	M. fortuitum (276, see also reference 519)
	M. microti (1246; 963)[e]
1930	
	M. thamnopheos (53)[f]
	M. marinum (52)
1920	
1910	
	M. chelonei (403; 126),[g] *M. lepraemurium* (1094; 769)
1900	
	M. phlei (842; 713)[h]
	M. bovis (1070; 585)
	M. paratuberculosis (552, 1188; 126)[i]
	M. avium (1104, 754; 244, see also [717])
1890	
	M. smegmatis (30; 713)
	M. tuberculosis (623; 712)
1800	
	M. leprae (species forma, 489, 712)[j]
1870	

TABLE 1—*Continued*

[a] Species are arranged chronologically according to the date of the initial description. With two exceptions, the mycobacterial species listed in this table are those having species status in *Bergey's Manual* edition 8 (196). Proposed mycobacterial taxa that are not recognized as having species status according to *Bergey's Manual* (196) and for which no synonymy has been proposed include: *M. anabanti* (130), *M. asiaticum* (1245), *M. aurum* (1175, 1164), *M. chitae* (1166), *M. duvalii* (1086), *M. engbaeki* (647), *M. gilvum* (1086), *M. lactis* (647), *M. obuense* (1170), *M. parafortuitum* (1174), *M. rhodesiae* (1171), *M. shimoidei* (1173a), and *M. thermoresistibile* (1163, 1164). Other as yet unsubstantiated taxa (see references 196 and 499) include: *M. album, M. azot-absorptum, M. brevicale, M. butanitrificans, M. cuneatum, M. gallinarum, M. hyalinum, M. methanicum, M. petroleophilum, M. rubrum,* and *M. sarni.*

[b] The first number(s) following each specific epithet is (are) the reference(s) describing that organism and/or its isolation. The reference just after the semicolon is that which establishes the presently accepted taxon.

[c] Common name: radish bacillus.

[d] Common name: Battey bacillus.

[e] Common name: vole bacillus.

[f] Lechevalier et al. (698), on the basis of an analysis for mycolic acids of the type strain, suggest that this species is probably a nocardia.

[g] See Table 2.

[h] Common names: timothy and hay bacilli.

[i] Common name: Johne's bacillus.

[j] Common name: Hansen's bacillus.

TABLE 2. *Certain accepted species of mycobacteria and their synonyms*

M. chelonei	(*abscessus*	849/1084, 1087
	borstelense	154/1084, 1087
	friedmanii	403; 1272/1084
	runyonii	151/1084, 1087)[a]
M. chelonei subsp. *abscessus* (*abscessus* 849/196, 666 *runyonii* 151/1172)		
M. chelonei subsp. *chelonei* (*borstelense* 154/196, 666, 1173)		
M. flavescens (*acapulcensis* 151/911, 1231)		
M. fortuitum (*giae*		282/449
	minetti	823, 918/449, 1248
	peregrinum	151/196, 666, 912
	ranae	676; 126/519, 912, 1085
	salmoniphilum	447; 992/attributed to Gordon [499])
M. gordonae (*aquae* sensu Bönicke [153] 417; 762, see also reference 418/1226, 1000)		
M. intracellulare (*Nocardia intracellularis* 275/996		
	brunense	604/196, 1002)
M. kansasii	(*luciflavum*	815; 768/196)
M. marinum	(*balnei*	882; 728/150
	platypoecilus	85/150, 806)
M. microti	(*muris* 1246; 1065/196)	
M. nonchromogenicum (*terrae* Tsukamura 1164/1165)		
M. paratuberculosis (*Johnei* 552; 392/196)		
M. phlei (*moelleri* 842; 244/196)		
M. scrofulaceum (*marianum* 1115, see also 917/1228, 1232, 1234		
	paraffinicum	292/196, 1002)
M. simiae	(*budapestae*	1244; 1009/1009
	habana	1196/1242, 1245a)
M. smegmatis	(*aquae*	417; 762, see also 418/449
	butyricum	923; 126/448
	friburgensis	644; 244/448
	lacticola	713/448)
M. terrae	(*novum*	1167/196)
M. ulcerans	(*buruli*	251; 250/1002)
M. xenopi	(*littorale*	777; 771/771, 778)

[a] References preceding bar (/): the first number(s) following each specific epithet is (are) the reference(s) describing that organism or its isolation or both; the reference following the semicolon is that which establishes the presently accepted taxon. The reference(s) following the bar (/) documents the synonym.

the carrying of a fetus and in the bearing of tumors. Although the specific mechanisms of accommodation or tolerance in these cases is not fully understood, they probably involve blocking antibodies (987a) and/or specific antigen-antibody complexes (1060a), either of which could interfere with the cytotoxic action of effector cells. The first stage in this process, the generation of toleragenic factors, occurs during protracted exposure to antigen, as in the case of animals immunized with complete Freund adjuvant (CFA) (26). It would appear reasonable to assume that the induction of tolerance might often be a necessary concomitant of mycobacterial infections.

It has recently been reported that many human subjects possess antibodies to *M. tuberculosis*. Was the production of these antibodies stimulated by *M. tuberculosis*? Or is their apparent specificity due to a cross-reaction? Does the arabinogalactan of wheat flour elicit antibodies cross-reactive with the arabinogalactans of *M. tuberculosis*, *Corynebacterium diphtheriae*, and *Nocardia asteroides*? Could such account for the reported widespread presence of antibodies against tubercle bacilli?

Over the years, a number of experiments have been designed to find out whether or not antibodies aid in recovery from tuberculous infection. One experiment reported herein (964) indicates that passively administered antibodies do not enhance recovery from experimental tuberculous infection. That experiment may also indicate that the introduction of an allogeneic serum into animals prior to and/or during tuberculous infection does not negatively affect tuberculosis in those animals. It is clear from a number of papers discussed by us that one should not equate "humoral immunity" with isolated immunoglobulins. What is important in the immune system is the interdependence of its components. Thus, the plasma cell arm, derived from the B component, not only produces antibodies and cytokines but also can exert a suppressor effect on the development of delayed hypersensitivity. On the other hand, the T-cell component of the immune system, required for the development of delayed hypersensitivity, exerts regulatory effects on the synthesis of antibodies. Interacting with each of these arms is the macrophage. Kostiala (648–650) has offered a prototype of the kinds of experiments that might reveal the workings of the immune system in the progress of, and in the recovery from, tuberculosis. Those experiments, coupled with data derived from such in vitro studies as (i) T cell, B cell, and macrophage behavior, using a Mishell and Dutton system (829), and (ii) delineation of the role of antibodies and lymphokines in phagosome-lysosome fusion and other intramacrophagic differentiative processes (Armstrong and Hart [51]), etc., could go a long way towards giving us more insight into mycobacterial infections.

It is hoped that future reviews of *Mycobacterium* may detail many mycobacterial products whose structures are known, as well as the structures of the host molecules whose appearance those products induce. In the present review, only a few such mycobacterial products have been reported. To date, other than immunoglobulins, few host response molecules have been characterized, and even the means by which the recovering animal disposes of mycobacteria remains to be discovered.

TAXONOMY: IDENTIFYING MYCOBACTERIA

Since this paper was submitted for publication, there has been published an exhaustive review, "Taxonomic Criteria for Mycobacteria and Nocardiae," by Bradley and Bond (177). The reader interested in the fundamental principles of mycobacterial taxonomy is referred to that paper.

In acquainting ourselves with the problems involved in sorting out strains of mycobacteria, we were impressed with the order brought to the genus *Mycobacterium* by the common sense of Runyon over a period of more than two decades (994–1002). His illuminating work has been augmented by that of Juhlin (568), of Tsukamura et al. (1172) and, more recently, of several groups and international cooperative committees (666, 669, 670, 1231). Already in 1968, Tsukamura and Mizuno (1169) had examined 97 characters in 754 strains of mycobacteria and concluded that a "hypothetical mean *Mycobacterium*" (HMM) could be prepared for *each* species using a numerical classification system. They proposed to define a species as a group of strains showing a mean S value [S value between two strains is a simple matching coefficient: $S(\%) = (Ns/Nd + Ns) \times 100$, where Nd is the number of characters showing different code symbols (+−), and Ns is the number of characters showing like code symbols (++ or −−)] of 90% or more to, for example, HMM^a, and showing mean S values of 89% or less to other HMMs such as HMM^c, HMM^d, etc. By this method *M. tuberculosis* and *M. bovis* are one species. The authors present their data clearly, acknowledge the contributions of others to their handling of problems in numerical taxonomy and discuss the enormity of the task of handling many strains and the usefulness of the hypothetical mean concept in such a task.

A recent paper by Tsukamura offers valuable

TABLE 3. *Formulation of mycobacterial taxa according to twelve properties examined by Kubica (665)*

(A) Twelve properties[a]

(1) Rate of growth (S = slow; F = fast)
(2) Secretion of niacin
(3) Reduction of nitrate ($NaNO_3$)
(4) Semiquantitative test for hyperproduction of catalase (column of gas bubbles, >45 mm)
(5) Stability of catalase to 68°C, 20 min
(6) Carotenogenesis constitutive (scotochromogenic)
(7) Carotenogenesis photoinducible (photochromogenic)
(8) Hydrolysis of Tween 80 after 10 days
(9) Reduction of tellurite ($KTeO_2$), 3 days
(10) Growth on media containing 5% (wt/vol) NaCl
(11) Hydrolysis of tripotassium phenolphthalein disulfate by arylsulfatase, 3 days
(12) Growth on MacConkey agar

(B) Formulae for 17 mycobacterial taxa and 1 species complex[b]

M. avium: S (5) [7] [9] / 3 4 6 8 10 12

M. bovis: S / 2 3 4

M. chelonei: F 4 (5) [8] [9] (10)[c] 11 (12) / 2

M. flavescens: S 3 (4) 5 (6) 8 [9] (10)

M. fortuitum[d]: F (3) (4) 5 [8] [9] 10 (11) (12) / 2

M. gastri: S — [12]

M. gordonae: S (4) (5) 6 (8) / 3 9 12

M. intracellulare: S (5) (9) [12] / 3 4 6 7 8 10 11

M. kansasii: S (3) (4) 5 (7) 8 / 2 6 12

M. marinum: S [2] 4 [5] (7) (8) / 3 10 11

M. phlei: F (3) 4 5 6 8 [9] 10

M. scrofulaceum: S [3] (4) 5 6 / 9

M. smegmatis: F 3 (4) (5) 8 (9) 10 / 11

M. terrae complex[e]: S (3) 4 (5) (8) / 6 7 10 12

M. triviale: S 3 (4) 5 8 10 [11] / 2

M. tuberculosis: S (2) (3) [8] / 5 6 9 10

M. vaccae: F (3) 4 5 [6] 7 8 (9) [10] [11]

M. xenopi: S (5) 6 [11] / 9

[a] Additional properties, examined by the International Working Group on Mycobacterial Taxonomy, which could supplement these tests, include a presumed hydrolysis of β-galactosides (1232a) and resistance to isoniazid, to thiophene 2-carboxylic acid hydrazide, to hydroxylamine, and to *p*-nitrobenzoate (1232b).

[b] Key for reading formulae. When number, N, is unboxed, N = 100% strains tested was positive (e.g., 11 in *M. chelonei*); (N) = 70 to 99% strains tested, positive (e.g., (5) in *M. avium*); [N] = 15 to 69% strains tested, positive (e.g., [7] in *M. avium*); $\bar{N}$ = 0.4 to 14% strains tested, positive (e.g., $\bar{3}$ in *M. avium*). Absence of N = 100% strains tested, negative (e.g., 11 absent in *M. avium*).

[c] *M. chelonei* subsp. *chelonei* fails to grow in 5% NaCl.

[d] *M. fortuitum* includes strains designated as *M. peregrinum*.

[e] *M. terrae* complex includes *M. terrae*, *M. nonchromogenicum* and *M. novum*.

data on the slow-growing species of mycobacteria (1168a) and points out the near identity of *M. scofulaceum*, *M. avium*, and *M. intracellulare*, a matter in which we agree and which sooner or later will bear directly on the taxonomy of acid-fast Hansen's bacilli.

Kubica (665) has performed the great service of presenting the 12 properties that are used by many mycobacteriologists for identifying members of the genus. These are summarized in Table 3 (see also Summary). An examination of this table reveals that *M. bovis* can be written as $\frac{S}{2\,3\,4}$. This translates: a slowly growing taxon in which niacin is secreted only by 0.4–14% of strains tested. A similar fraction can reduce nitrates and show catalase activity. On the other hand the closely related *M. tuberculosis* = $\frac{S\,(2)\,(3)\,[8]}{5, 6\ 9\ 10}$. An examination of Table 3 reveals that *M. tuberculosis* is a slowly

TABLE 4. *Mycobacteria reported to cause disease in human subjects*

Mycobacterium	Related disease(s)	Source[a]
M. africanum	Pulmonary	225, 226, 474, 527
M. avium	Pulmonary	173, 245, 312, 346, 515, 772
	Extrapulmonary:	
	Disseminated	360, 683, 1032
	Lymphadenitis	312, 332, 345, 615, 726, 772
	Associated with silicosis	543, 1278
	Meningitis	312, 887
	Ocular	1222
	G. R.[b]	366, 672
M. bovis	Pulmonary	88, 472, 584, 589, 692, 716, 755
	Extrapulmonary:	
	Disseminated	480, 584
	Lymphadenitis	230
	Meningitis	460
	Bone and joint	230
	Miscellaneous	584
	G. R.	230, 367, 472, 907, 1259
M. bovis BCG	Extrapulmonary:	
	Disseminated	766, 774
M. chelonei	Pulmonary	475, 1173
	Extrapulmonary:	
	Injection and abrasion abscesses	160, 419, 535, 849, 913
	Disseminated	468
	Lymphadenitis (cervical)	854, 855
M. fortuitum	Pulmonary	92, 263, 320, 877, 950, 1208
	Extrapulmonary:	
	Corneal	720, 1186, 1187, 1288, 1311
	Disseminated	363
	Injection and abrasion abscesses	115, 214, 252, 276, 487, 892, 901, 1053, 1199, 1248
	Miscellaneous	504, 1062
M. intracellulare	Pulmonary	262, 269, 368, 608, 719, 1293
	Extrapulmonary:	
	Disseminated	275, 294, 628, 719, 1010, 1210
	Lymphadenitis	693, 726
M. kansasii	Pulmonary	139, 235, 236, 551, 608, 719, 927, 1303
	Extrapulmonary:	
	Disseminated	348, 473, 484, 803, 1281
	Miscellaneous	431, 434, 503, 526, 719, 745, 902, 1223
	G. R.	1281
M. leprae (species forma)	Extrapulmonary	
	Tuberculoid ↔ lepromatous leprosy	514, 565
M. lepraemurium	Extrapulmonary	
	Nodular infections or skin and lymph nodes	770
M. marinum	Extrapulmonary:	
	Cutaneous granulomas (including "swimming pool granulomas")	98, 254, 264, 729, 847, 852, 928, 1005, 1075, 1116, 1215, 1310
	Sporotrichoid	1, 11, 212, 299, 378, 558, 1277
	Miscellaneous	382, 461, 558, 580, 1264, 1273
M. scrofulaceum	Pulmonary	1275
	Extrapulmonary:	
	Lymphadenitis (cervical)	133, 942, 1275, 1276
	Miscellaneous	848
M. simiae (*habana*)	Pulmonary	1195, 1242
M. szulgai	Pulmonary	776
	Extrapulmonary:	
	Lymphadenitis (cervical)	776
	Joint	776
M. terrae	Extrapulmonary:	
	Disseminated	247
M. triviale	Extrapulmonary:	
	Arthritis	344

TABLE 4—*Continued*

Mycobacterium	Related disease(s)	Source[a]
M. tuberculosis	G. R.	477, 973
M. ulcerans (*buruli*)	Extrapulmonary: Chronic skin ulcerations	28, 94, 197, 250, 251, 259, 260, 307, 361, 540, 687, 737, 746, 925, 965, 1069, 1160, 1221
M. xenopi	Pulmonary	149, 296, 311, 343, 347, 372, 778, 805, 980

[a] References cited are those in which specific cases were examined by the author(s). Only general reviews are given for the widely investigated species, *M. tuberculosis* and *M. leprae*. References were selected to demonstrate the variety of disease involvement reported in the literature and are not meant to be inclusive.

[b] G. R., General references. Other general references include 234, 235, 284, 365, 366, 383, 391, 515, 726, 773, 926, 973, 994, 995, 998, 1001, 1056, 1186, 1254, 1276.

growing taxon, 70 to 99% of whose members secrete niacin, 100% produce catalase, etc. The uninitiated reader might ask: Are these 12 properties peculiar to mycobacteria? Of course not. Their presence or absence can equally well be determined for *Corynebacterium*, *Nocardia*, and a wide variety of other bacteria. However, for nonsporeforming, gram-positive bacteria exhibiting superficial ropelike structures (see Fig. 6) and mycobacterial acid-fastness (as opposed to other types of acid-fastness), these 12 properties serve to delineate the species listed in Table 1.

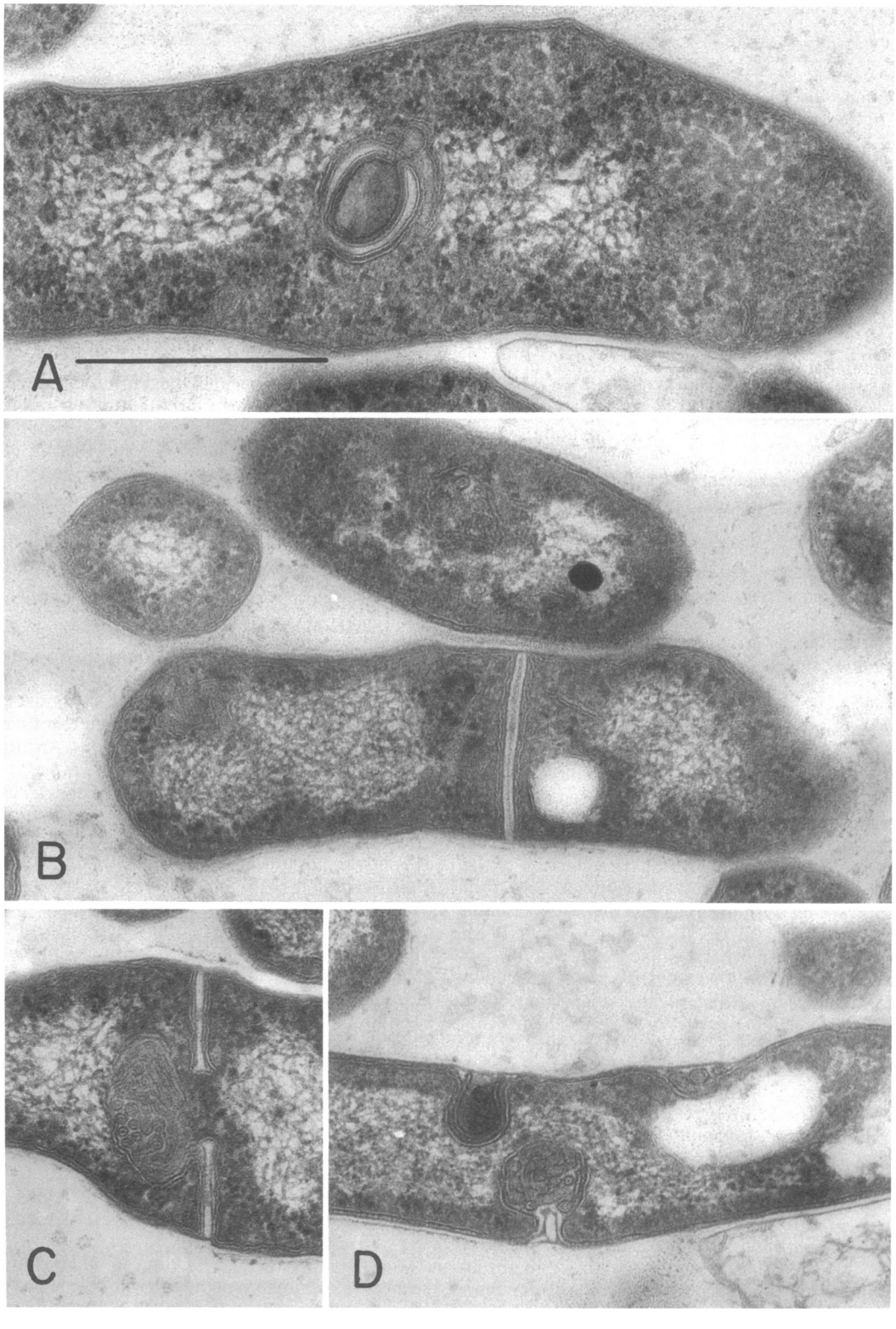
In Table 4 are listed those mycobacteria that, at one time or another, have been associated with human disease. Many of them are not primarily found in man. One entry, *M. lepraemurium*, has been included because it is from a provocative paper that beautifully points up the difficulties in dealing with so-called noncultivable species. The data in Table 4 are taken from a more comprehensive table we have prepared on the host ranges of mycobacteria. The use of a term such as "host range" is a reflection of the zoocentric (mostly anthropocentric) view that interacting with animals is a prime function of mycobacteria. In truth, these microbes, of remarkable biosynthetic capacities, have in their dispersions down through time got caught up in nooks, crannies, and niches where several of them have lost, to varying degrees, their independence. However, studying them only as producers of disease would yield as narrow a view of their capabilities as would studying them only for the 12 properties listed in Table 3.

Eventually, taxonomists will probably find that the *Corynebacterium*, *Mycobacterium*, and *Nocardia* (CMN) group of microorganisms is best treated as a single genus. This decision will no doubt come as a result of insights gained by an examination of the comparative biology of CMN. Such will require (i) direct examination of the ranges of colonial and cellular morphology, (ii) growth and metabolism (best examined through comparison of mutant strains, where possible, augmented by recombinational genetics), and (iii) various biosynthetic capacities leading to the synthesis of distinctive products. Information so gained can be supplemented with (iv) studies of the interaction of particular bacteria and their viruses and (v) their interaction with animal hosts as sources of antigen (structural components such as mureins or peptidoglycans, enzymes, toxins, etc.). The present review supplies much of this information for *Mycobacterium*.

THE MYCOBACTERIAL CELL

Much is known about the chemistry of various compounds produced by mycobacteria, and microbial anatomists, microbial biochemists, and geneticists now need to put this all together in terms of a growing *Mycobacterium*. An ultrathin section of actively growing *M. tuberculosis* $H_{37}Rv$ is shown in Fig. 1. Mycobacteria are nonmotile, nonsporeforming, pleomorphic, gram-positive bacilli. Mycobacteria, growing on solid media, pile up to form smooth or rough, often wrinkled, soft and buttery, powdery to waxy (see Fig. 16), transparent to opaque colonies (e.g., see Fig. 1 of reference 1025 and Fig. 1 of reference 898). Sometimes, in old cultures, mycobacteria show an inhibition of postdivisional cell separation that is apparent as filamentous growth and, less commonly, as branching. Runyon has pointed out that, on the basis of their tendencies to form aerial filaments, mycobacteria range from those, such as *M. tuberculosis* and *M. bovis*, which show no filamentation to those forming rudimentary filaments (*M. xenopi*), to those that show filamentous extensions from their colonies (*M. fortuitum*), with fragmentation to the obviously filamentous *M. farcinogenes*, which rarely shows fragmentation (999).

Forty-five years ago, Kahn, in an elegant series of experiments using microdrops, showed that the ancestral strain of *M. tuberculosis*,


A
B
C
D

$H_{37}Rv$, underwent fragmentation to coccal forms, and rods developed from some of these cocci (570). In Fig. 2B is shown a cell of $H_{37}Rv$ from an actively growing culture in which two of the compartments show no signs of viability. The terminal one, however, obviously has healthy nucleic acid, dense cytoplasm, polysomes, and a fatty inclusion. The overall dimensions of this cytoplast, 1.0 by 0.3 μm, are equivalent to those of Kahn's coccal bodies. In some species, such as *M. avium*, growth is characterized by an increase in the number of cytoplasts within a common cell wall, with periodic fragmentation to release coccoid cells. Maffucci (754) observed these peculiarities of *M. avium* in 1892. They were rediscovered and carefully described as a "life cycle" for *M. avium* by Brieger and Fell some three decades ago (185). McCarthy (800) has reported the use of two mutants of *M. avium* for studying cell growth and division, employing photomicrography, electronic counting and size distribution analyses, and viability and protein determinations. The mutants were colonial types termed transparent (T) and opaque (O). T was virulent; O was not (898), 1025; see also [48a]). T was resistant to more drugs than O and formed a smaller cell mass than O. The initial inoculum was sized by passage through a 1.2-μm-pore-size filter (type MF, Millipore Corp.), and synchronous growth was obtained. This very beautiful paper establishes that growth of *M. avium* involves an increase in deoxyribonucleic acid (DNA), protein, and cell mass, concomitant with a lengthening of the cell body. With fragmentation, the DNA and protein decreased to preelongation ratios. This fluctuation between coccal forms and pleomorphic rods is not uncommon in the CMN group (e.g., in the genus *Nocardia* [114] and in *C. diphtheriae* [293]). One of the most interesting findings of McCarthy was that the virulent T mutant produced one-twentieth as many viable units as the O mutant. Would these units, nonviable in vitro, have been viable in vivo? In Fig. 3 are shown small cells of *M. intracellulare* (a close relative of *M. avium*) in association with longer ghost cells.

Although most of the studies of transitions from rods to coccal forms and vice versa in mycobacteria have been with facultatively parasitic mycobacteria, Chang and Andersen have noted the occurrence of chains of tiny acid-fast coccal forms during the growth of the *obligately* parasitic *M. lepaemurium* in mouse macrophages (231).

Acid-Fastness

Acid-fastness is the capacity of biological materials to form acid-stable complexes with certain arylmethane dyes. Such materials contain dye complexes that are not decolorized following exposure to acidic ethanol or mineral acids. This general property exists in a variety of entities, including spores of a number of fungi (1294), the spores of *Bacillus cereus* (1297), human sperm (125), the embryophores of *Taenia saginata* (914), the hooklets of *Taenia echinococcus* (194), corynebacteria and/or certain of their inclusions (929a, and our unpublished data), tubercle bacilli (623), leprosy bacilli (1151), keratin (C. A. Fisher, unpublished data), nuclear DNA (as in the Feulgen reaction), and chitin following exposure, in situ, to mild oxidation (916). In each case the biological poduct responsible for stably combining with the dye is apparently different: for example, the capacity for acid-fastness of the spores of *B. cereus* is associated with β-hydroxybutyrate and can be removed by extracting the spores with chloroform (1297); that of leprosy bacilli can be extracted with pyridine whereas that of tubercle bacilli cannot (377). The *capacity for acid-fastness* of various mycobacteria can be removed with alkaline ethanol (377). It is clear, then, that the term acid-fastness, in order to take on meaning, needs be qualified according to the chemistry of each potentially acid-fast material.

Mycobacterial acid-fastness. Mycobacterial cells that have taken up fuchsin (triaminotriphenylmethane chloride; pararosanilin), crystal violet (hexamethylpararosanilin chloride), or auramine O (tetramethyldiaminodiphenyl ketoimine) in phenol-water (as carbol fuchsin, carbol crystal violet, or carbol auramine O)

FIG. 1. *Sections of cells of actively growing M. tuberculosis $H_{37}Rv$ 102. (A) Mesosome within nucleoid showing central homogeneous matrix of intralamellar spaces. See sections, The Mycobacterial Cell and Nucleoid in text. (B) A completed cross wall showing a distance of about 26 nm in width with an electron-transparent layer separating two cells which are still connected to the mother cell wall. Note membrane-bound vesicle and metachromatic granule. (C) A cross wall halfway completed showing continuity with plasma membrane and a large vesiculated mesosome. (D) The initiation of cross wall formation, showing large mesosomes at both sides of the cell. Note the vertical position of the mesosomes in relation to the initial cross wall. (All micrographs, ×78,000; bar = 0.5 μm.) Cells were fixed with OsO_4, dehydrated in ethanol, and embedded in liquid epoxy resin, D.E.R. 736 (The Dow Chemical Co.). The following micrographs (sectioned materials) were prepared in same way. See also Tables 1, 2, and 3.*

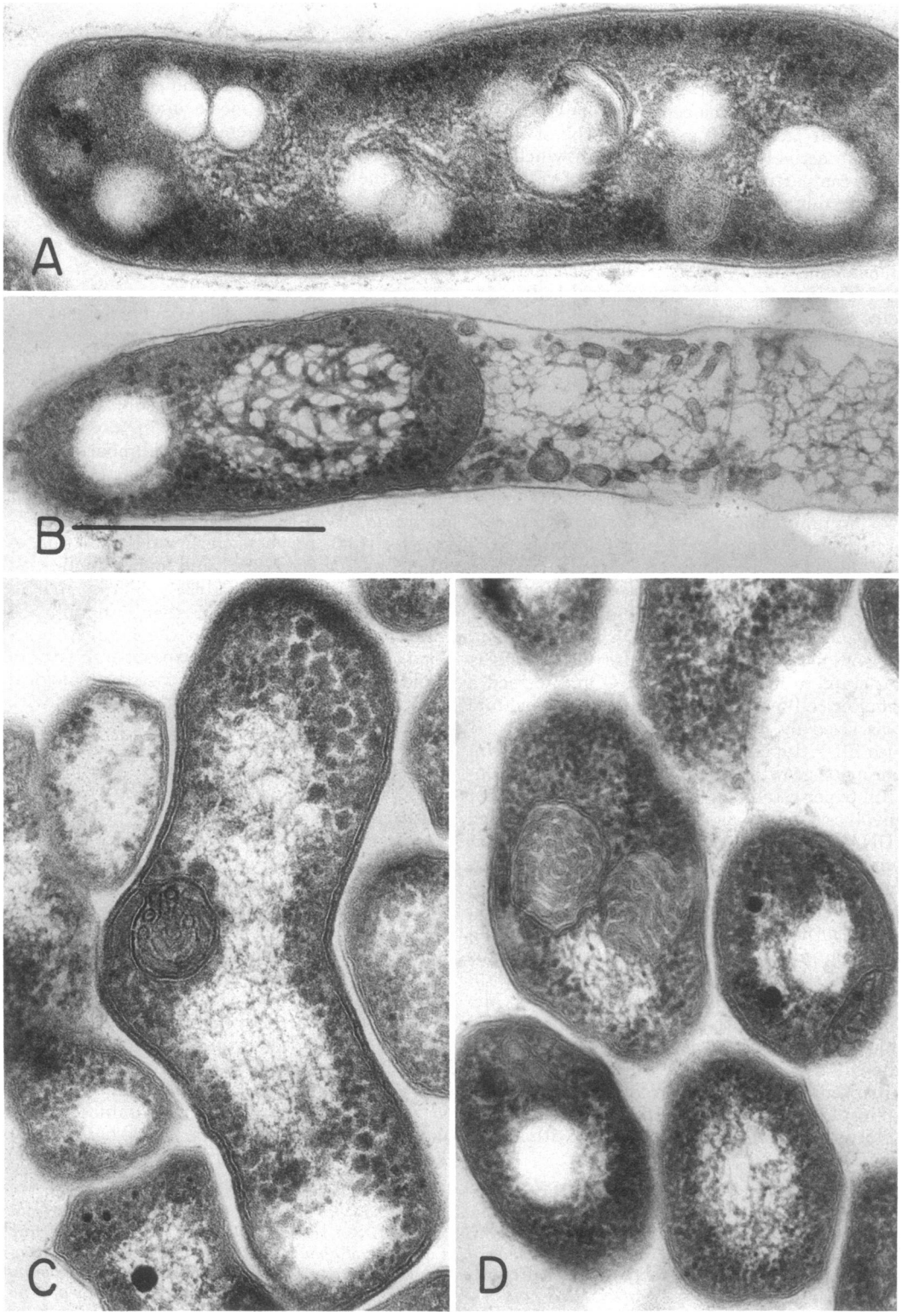


FIG. 2. *(A) Section of M. aurum cell showing vacuole-like fat inclusions enclosed by a membranous structure. (B) Section of an actively growing cell of M. tuberculosis $H_{37}Rv$ 102 showing viable compartment within lysed old mother cell. Note lack of classical cross wall formation. (C) and (D) Sections of actively growing cells of M. tuberculosis $H_{37}Rv$ 102 showing vesiculated type of mesosomes. Continuity of cytoplasmic membrane is clearly visible. (All micrographs, ×78,000; bar = 0.5 μm.) See also discussion of The Mycobacterial Cell. See Tables 1, 2, and 3.*

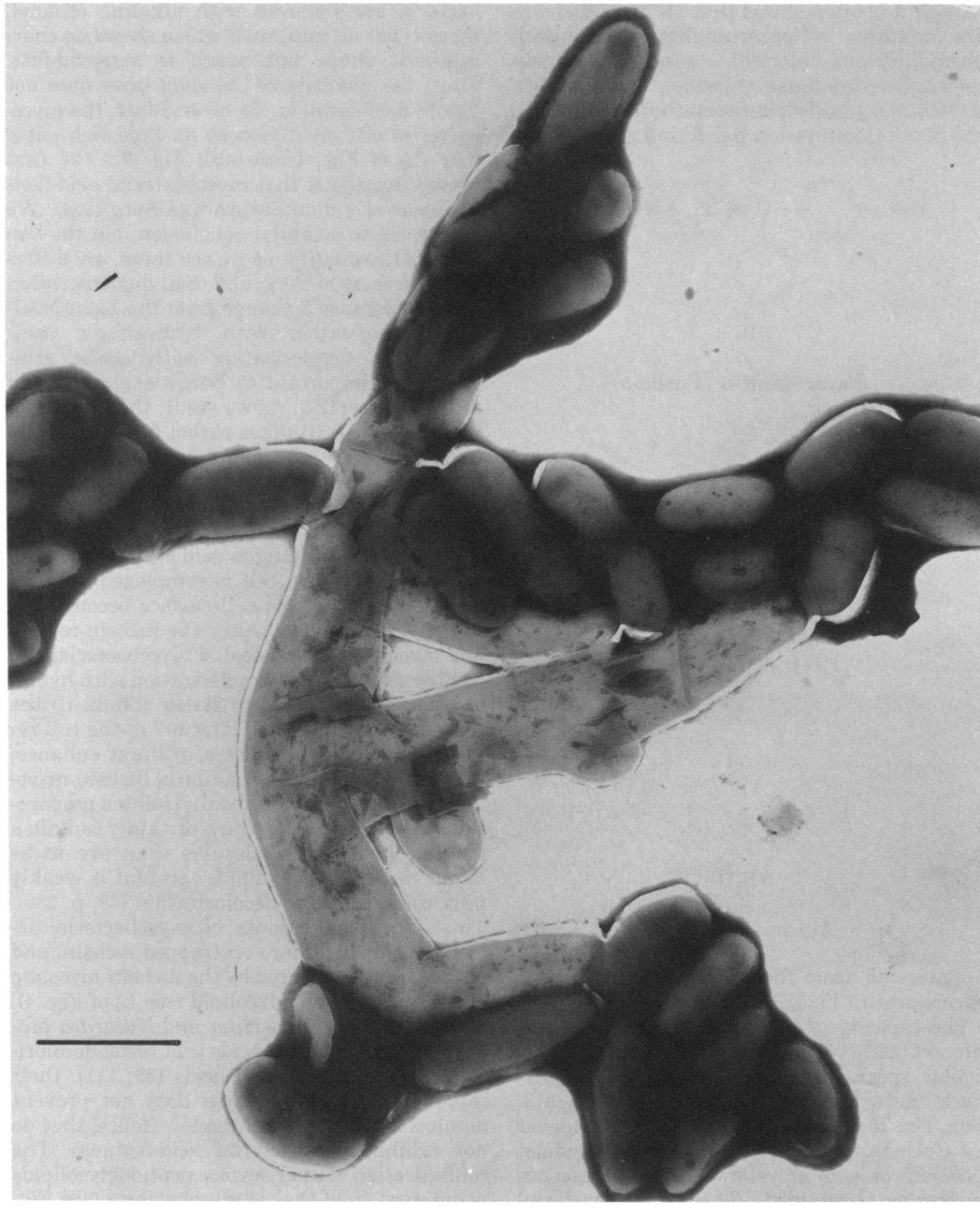
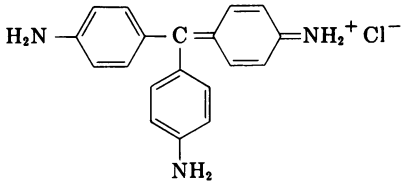


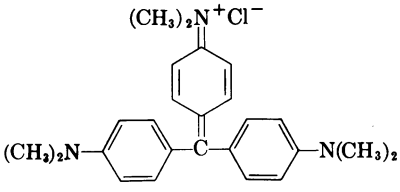
FIG. 3. *Ammonium molybdate-stained cells of M. intracellulare from a 1-month-old culture. An important property of M. intracellulare, M. avium, and certain other mycobacteria is the formation of small cellular units in association with large ghost cells. Note fibrous structures (see text under peptidoglycolipid) which, in a manner, traverse the cell wall of the large empty cell. Small electron-dense cytoplasmic inclusions are discernible in the smaller, intact cells. (×20,000; bar = 1 μm.) This and subsequent micrographs (negatively contrasted materials) were pepared from grids stained with 2% ammonium molybdate. See Tables 1, 2, and 3.*

usually resist decolorization by acidic ethanol as applied in the Ziehl-Neelsen stain. In a strict sense, mycobacterial acid-fastness has to be defined as a resistance to decolorization with acid alcohol, since a number of corynebacteria and nocardiae grown on specific media (containing glycerol) are resistant to decolorization by dilute (1 to 10%) mineral acid (491). (Al-

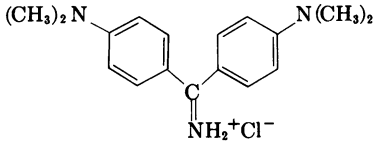
though it is often stated that all basic fuchsins are mixtures of pararosanilin [triaminotriphenylmethane chloride], rosanilin [triaminotolyldiphenylmethane chloride], and magenta II [triaminoditolylphenylmethane chloride], the Stain Commission has found many of the



Pararosanilin (Fuchsin)



Crystal Violet



Auramine O

commercial basic fuchsins to be fairly pure pararosanilin [725].)

The capacity of these anionic compounds to interact with various naturally occurring molecular species, including a number of polymeric ones, has long been known (725, 813, 899). The resultant acid-resistant cells appear red (fuchsin retained) or purple (crystal violet retained) or exhibit yellow-green fluorescence (auramine O retained). This is mycobacterial acid-fastness, regarded by many as the hallmark of mycobacteria. Fuchsin-stained, acid-fast mycobacteria exhibit a marked brilliance of color. Mycobacterial cells which have been broken open and then subjected to the acid-fast stain are only weakly acid-fast, lack brilliance of color and, undoubtedly because of the obscuring counter stain (124), have long been called non-acid-fast (624). If the outer walls of mycobacteria are removed with alkaline ethanol, there is left an intact cell which shows its characteristic shape but which is non-acid-fast. Thus, the integrity of the rigid layer does not assure acid-fastness. To be acid-fast, the mycobacterial cell must possess its lipid rich outer coat (L_2 of Fig. 4; see also Fig. 5). The first person to suggest that mycobacterial acid-fastness was of a dual nature was Berg (124). We have come to a similar conclusion, but the two parts of the duality, as we see them, are different from those of Berg, and their demonstration does not require a change from the Ziehl-Neelsen decolorization with hydrochloric acid-ethanol to decolorization with acetic acid-ethanol so important to Berg's explanation of acid-fastness (122). As we see it, the intact mycobacterial cell (i) takes carbol fuchsin into its interior and (ii) also binds fuchsin to the mycolic acid residues of the peptidoglycolipids of the outer cell wall (Fig. 4 and 5). Free mycolic acids bind fuchsin on a mole-for-mole basis (123), and the bonding is acid stable. Once the mycolic acid of the cell is complexed with an arylmethane dye, the cell surface becomes extremely hydrophobic. After the fuchsin-replete and mycolate-fuchsin-coated mycobacterial cell has been subjected to decolorization with hydrochloric acid-ethanol, two states obtain: (i) the fuchsin taken into the "interior" of the cell remains there and supplies a brilliant enhancement to (ii) the lightly staining fuchsin-mycolate complex of the outer wall. Isolated preparations of mycolic acids, which certainly contain a greater density of molecules than are to be found in peptidoglycolipids, present a weakly pink color following decolorization (59, p. 256). Thus, the acid-fastness of mycobacteria depends for its brilliance on trapped fuchsin, and the trapping is ensured by the fuchsin-mycolate of the outer peptidoglycolipid (see L_2 of Fig. 4). Although *Corynebacterium* and *Nocardia* produce plenty of mycolic acids that resist decolorization by dilute mineral acids (59, 111), their mycolic acid-bound fuchsin does not prevent decolorization by acidic ethanol. Hence, they do not exhibit mycobacterial acid-fastness. The configuration of their surface peptidoglycolipids is different from that of mycobacteria (see Fig. 22-26), and this leads us to suggest that the chemical constituents of the unique ropelike structures of the outer mycobacterial cell wall are essential for mycobacterial acid-fastness.

Ziehl-Neelsen stain. Most of what is known of the chemistry of the acid-fast reaction in mycobacteria has been derived from the study of the Ziehl-Neelsen stain, in which the dye employed for acid-stable binding is carbol

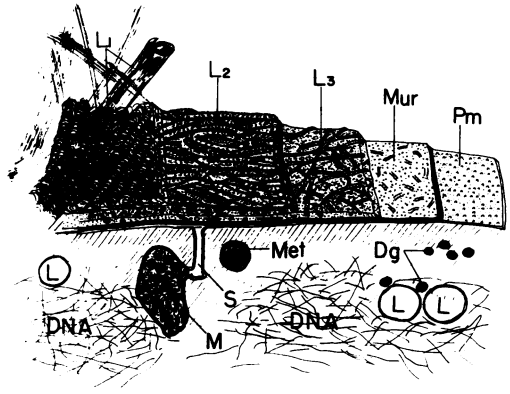


FIG. 4. *The mycobacterial cell wall as diagrammed from electron micrographic data considered in this review. Beginning with the plasma membrane (Pm) and coming past the murein or peptidoglycan (Mur), the underlying fibrous ropelike structures of the cell wall are shown as L_3 merging into the more wrinkled and superficial L_2 with which it is continuous. This material lies immediately under the sheetlike surface glycolipid or peptidoglycolipid (L_1). Contained within the Pm are shown a growing septum (S) with its vesiculated mesosome (M) associated with DNA of the nucleoid, metachromatic granule (Met), lipoidal bodies (L), and dense granules (Dg) (see Fig. 10C). For biological activity associated with Ls see Tuberculins and Other Mycobacterial Elicitins, as well as Delayed Hypersensitivity; for discussion of chemistry of these layers, see From the Cytoplasmic Membrane to the Peptidolipid and parts of Mycobacterium as Antigen. In Fig. 5, L_3, L_2, and L_1, as well as the murein or peptidoglycan layer, have been rendered visible by negative staining (5A) and freeze-etching (5B). Note: Although macromolecular structures such as L_2 and L_3 can be rendered visible, other surface components such as trehalose dimycolate (cord factor), acyl glucoses, etc., cannot.*

fuchsin. Lartigue and Fite (694) established the fact that, in *carbol fuchsin*, fuchsin and phenol are independently associated (no chemical modification of either compound) and confirmed the contention of Lamanna (684) that phenol (and, for them, detergents as well) enhances the penetration of fuchsin into lipids by rendering the fuchsin more lipid soluble and less water soluble. Since the time of Stodola et al. (1103), it has been held that mycolic acids per se were acid-fast. However, Richards (975) and Rich (972), as well as Yegian and Vanderlinde (1294), have regarded that as untrue, perhaps because thin films of mycolic acid were only faintly pink following exposure to acidic ethanol. The faintness of color was due, no doubt, (i) to the low degree of binding of dye when the lipoidal receptors for that dye were dispersed rather than ordered in the ultrastructure of the *Mycobacterium* and (ii) to the absence of the background color provided by intracellular dye (see above). The importance to acid-fastness of the way and extent to which mycolic acids are ordered about the mycobacterial cell wall is suggested by the report of Murohashi et al., who detected no correlation between mycolate yields and degree of acid-fastness of strains (865).

Interaction of dye and mycolic acid. In reviewing the properties essential for the acid-fastness of mycolic acids, Asselineau has pointed out that the mycolic acid must possess a free carboxyl group (methyl mycolate and mycolic alcohol were not acid-fast). The hydroxyl group must be present, but it could be esterified, as in acetates of mycolic acid (59, p. 255). Berg tested the interaction of a mycolic acid, leprosinic acid, with crystal violet after the blocking of its two carboxyl groups by methylation. No dye complexing occurred. In fact, complex formation was prevented even when the two carboxyls were free, but one of the hydroxyls had been blocked by acetylation. Free mycolic acid was found by Berg to bind crystal violet on a mole-for-mole basis. The dye-acid complex has a characteristic peak at 350 μm (123). This is a very different peak from that found with crystal violet and agar or nucleic acid (813). The mycolic acid-dye complex, as well as mycolic acid, was soluble in xylene, although the dye alone was not (123).

Ropelike structures, the integrity of the rigid layer of the cell wall, and acid-fastness. In this paper, we give the first documentation that all species of mycobacteria appear to possess surface ropelike structurs of peptidoglycolipid and that, from species to species, the pattern assumed by this material is similar (Fig. 6). The ropelike peptidoglycolipid seems to be an essential part of the duality (124) of mycobacterial acid-fastness. It furnishes the reacting mycolic acid residues required for producing the acid-fast arylmethane mycolate essential for blocking the exit of fuchsin taken into the cell during the staining process. The following findings indicate that a ropelike configuration per se is not necessary to the functioning of this peptidoglycolipid in acid-fastness. Incubation of cells of actively growing strain 607 (in Penassay medium [Difco] plus Tween 80) in the presence of ethylenediaminetetraacetic acid (EDTA) (200 μg/ml) and lysozyme (100 μg/ml) for 15 h permits the elongation of the cells showing a marked absence of observable ropelike structures. *These cells*, nevertheless, *are strongly acid-fast* (see Fig.

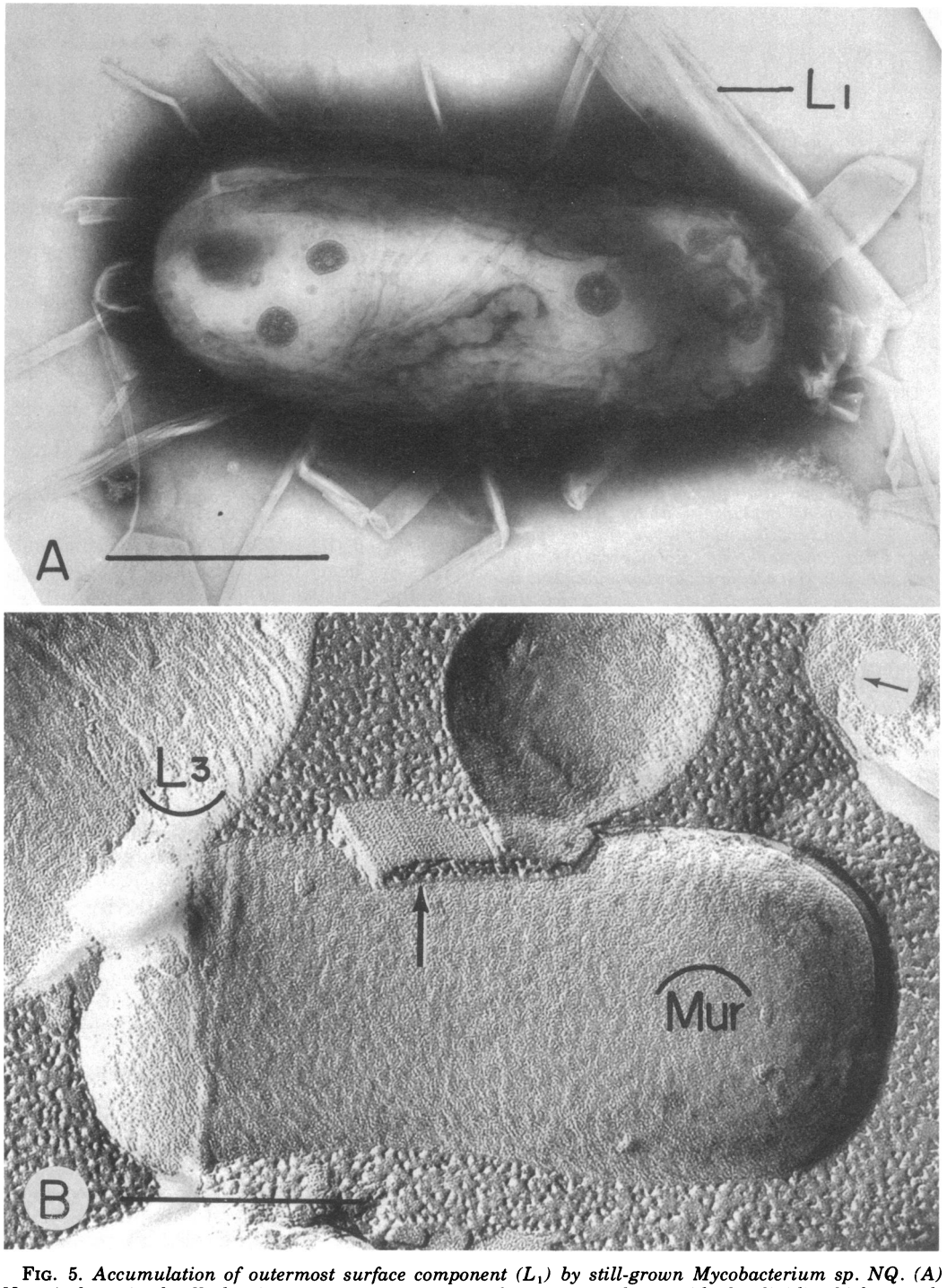


FIG. 5. *Accumulation of outermost surface component (L_1) by still-grown Mycobacterium sp. NQ. (A) Negatively stained cell showing outer encasing material (see text under peptidoglycolipid) which extends beyond the cell as ribbon-like appendages. Note that characteristic ropelike structures are covered up, although polyphosphate granules may be seen within the cell. (×65,000; bar = 0.5 μm.) (B) Micrograph of freeze-etched Mycobacterium sp. NQ. When the ribbon-like structures shown in (A) are shaken away from the cell, ropelike structures are exposed. These can be visualized by negative staining as shown in Fig. 24I. They are characteristic surface structures of mycobacteria and differ in size from analogous structures found in Nocardia (Fig. 26) and Corynebacterium (Fig. 25). The uncircled arrow indicates a fracture through L_1 revealing in profile the ropelike structures of L_2 and L_3 adhering to the rigid murein layer, which is exposed as a smooth background. The direction of the shadowing is indicated by the circled arrow. (×140,000; bar = 0.25 μm.) For a discussion of this component of mycobacterium as receptor for bacteriophage see Host Cell Receptors and Bacteriophage-Induced Receptor-Destroying Enzymes. For general chemical structures see Peptidoglycolipids or Mycosides. For details regarding production of mycosidic mycobacterial casements, see reference 614.*

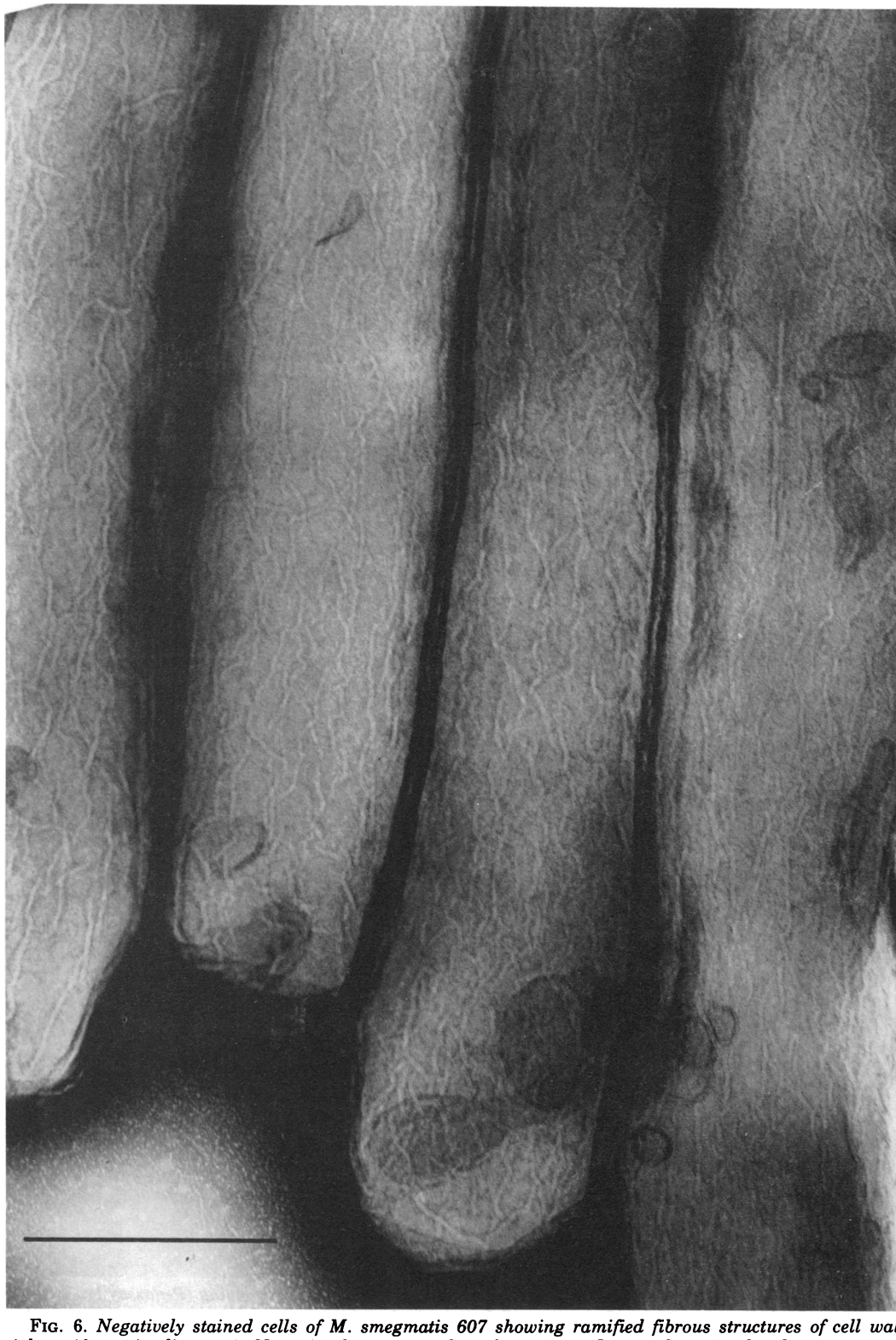


FIG. 6. *Negatively stained cells of M. smegmatis 607 showing ramified fibrous structures of cell wall (about 10 nm in diameter). Note simple mesosomal configurations. In mycobacteria that have grown as pellicles or colonies, or in animal tissues, where the outermost layer of peptidolipid or peptidoglycolipid has not been disturbed, ropelike structures are only faintly evident. Subjecting such cells to sonic vibration or repeated washing eliminates much of the outer layer and reveals the ropelike structures. Figures 22–24 establish the fact that, although these ramified, fibrous patterns appear characteristic of the genus Mycobacterium, they are not very distinctive from one species of Mycobacterium to another. (×78,000; bar = 0.5 μm.) See text: Nonpeptidoglycan Amino Acids and Superficial Glycolipids and Peptodoglycolipids: Ropelike Patterns. See also Tables 1, 2, and 3.*

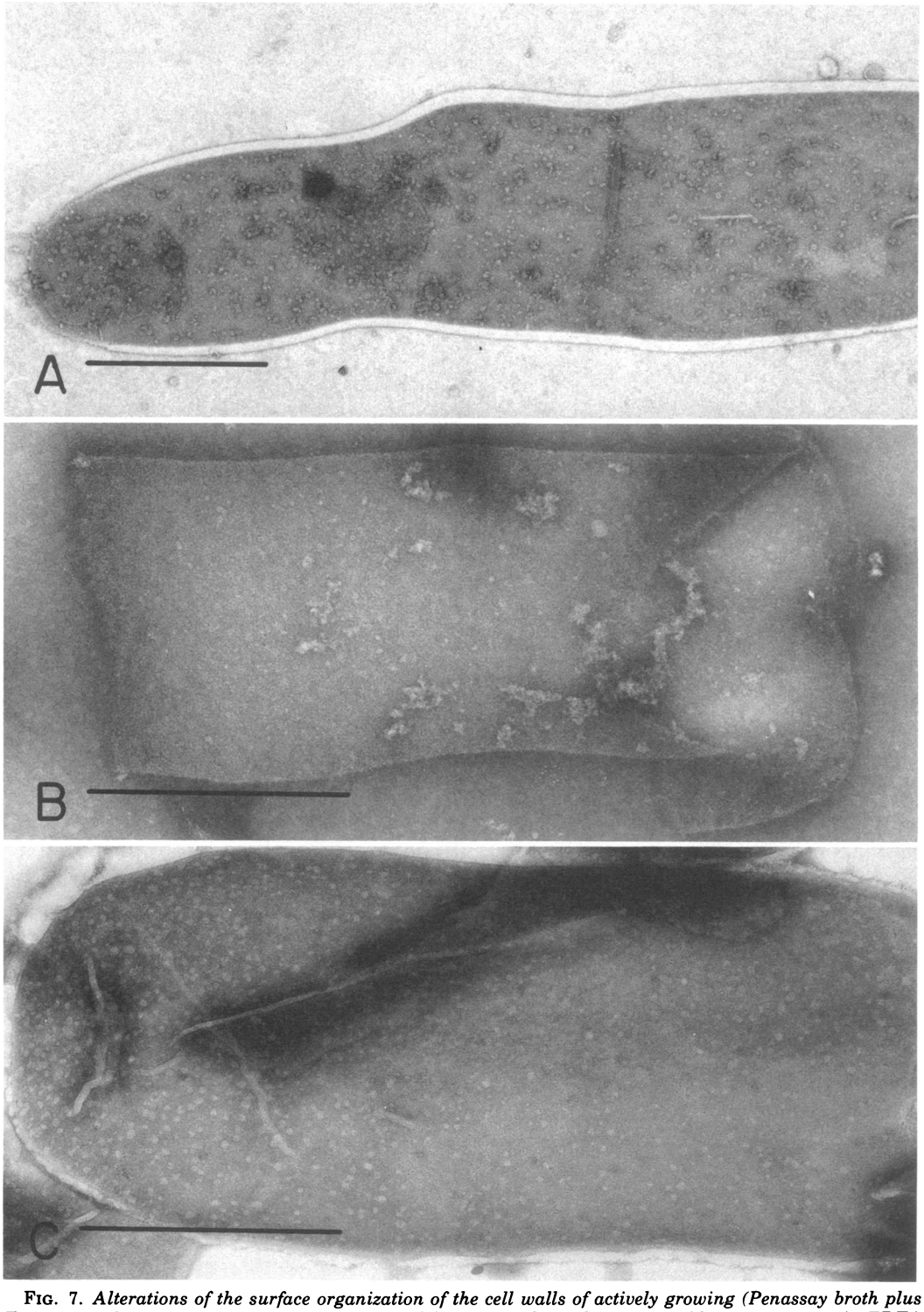


FIG. 7. *Alterations of the surface organization of the cell walls of actively growing (Penassay broth plus Tween 80) M. smegmatis 607 in relation to acid-fastness and pyridine-extractable components. (A) EDTA (200 μg/ml) and lysozyme (100 μg/ml) were added to the growth medium, and incubation was continued for 15 h. Negatively stained cell shows an absence of fibrous structures. These cells were acid-fast at 15 h but non-acid-fast at 24 h (see text). (×27,000; bar = 1 μm.) (B) Cells treated with 0.5% (wt/vol) KOH-ethanol (see also Fig. 8B) for 4 h lose their acid-fastness. (×78,000; bar = 0.5 μm.) (C) Cells were suspended in pyridine for 4 h and then prepared for negative staining. Most of the ramified structures disappeared. Many ringlike surface particles are apparent. Cells are acid-fast. Their enhanced permeability to fuchsin is discussed in the section on phosphatidylinositol polymannosides. (×78,000; bar = 0.5 μm.) See Tables 1, 2, and 3.*

7A). Further prolonged incubation (total, 24 h) results in a loss of acid-fastness. Extraction of surface lipids with 0.5 to 1% (wt/vol) KOH-ethanol for 4 h results in complete loss of acid-fastness and of ropelike structures (Fig. 7B and 8B). This probably means that, although the ropelike configuration per se is not essential to acid-fastness, the *substance which assumes that configuration is essential.*

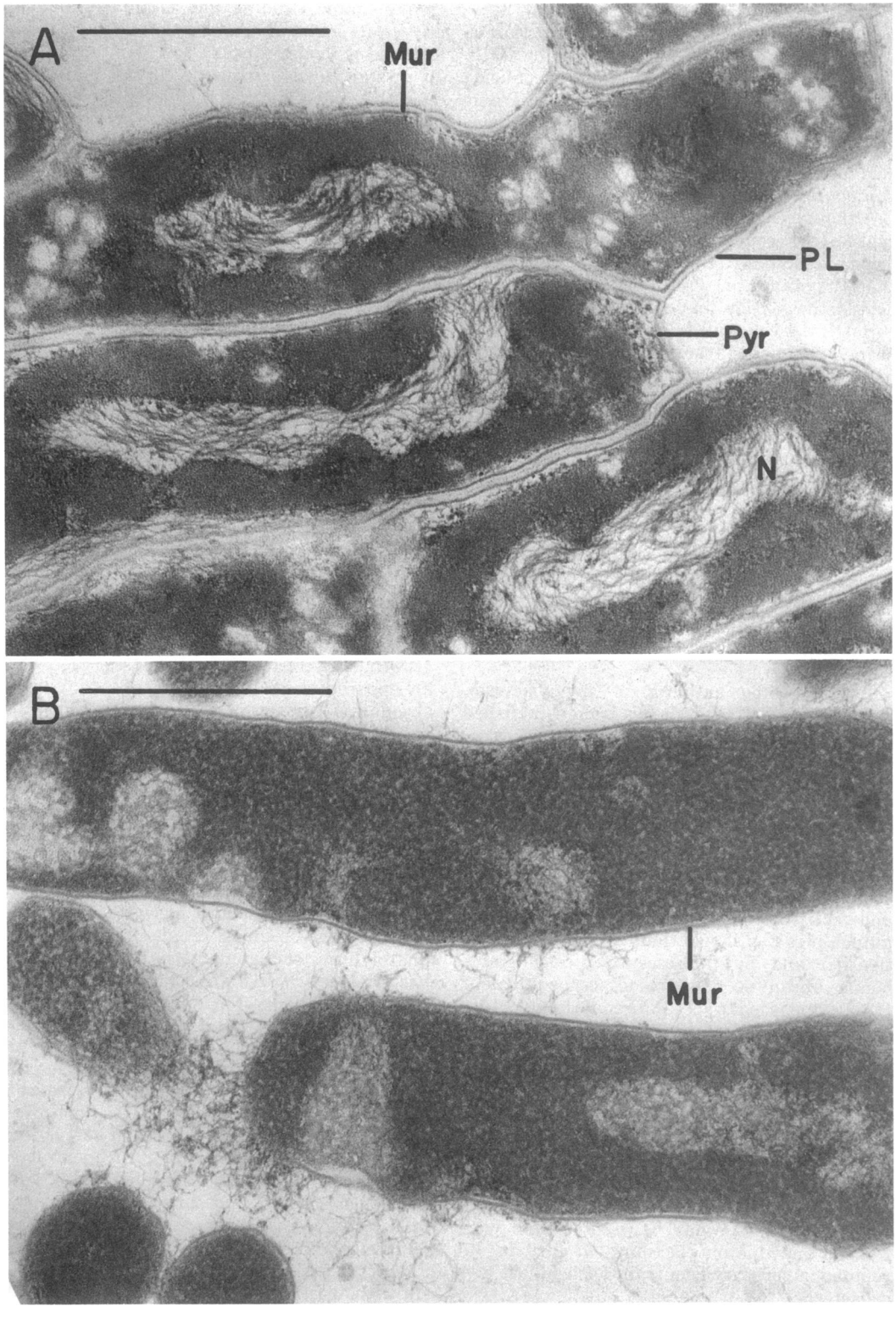
Although Koch's early demonstration that acid-fastness could be destroyed by grinding the dried bacilli in an agate mortar (624) suggested that the intact bacterial cell was needed for acid-fastness, cells that contain open channels through the cell wall to the cytoplasmic membrane (pyridine-extracted cells [Fig. 8A]) remain acid-fast. This, plus the fact that delipidated cells are not acid-fast, leaves little doubt that in the dual nature of acid-fastness (i) the brilliance of the stain is attributable to trapped intracellular fuchsin and (ii) the trapping of the fuchsin is the result of a barrier furnished by mycolate-fuchsin complexes of the peptidoglycolipid of the outer cell wall.

Interference with the synthesis of mycolic acids as, for example, in the treatment of mycobacteria with isonicotinic acid hydrazide (INH) leads to non-acid-fastness (see section, Isonicotinic Acid Hydrazide and Mycolic Acid Synthesis).

Fluorochrome staining of mycobacteria. Over the past 15 years it has been established that fluorescent staining of *Mycobacterium tuberculosis* (482) with tetramethyldiaminodiphenylketoimine (auramine O) gives a higher yield of positive findings than does staining with carbol fuchsin. This proves true both for bacilli in sputa (979) and in histological sections (466, 1289). In fact, a comparison between Ziehl-Neelsen staining, auramine O staining, and cultivation of material from infected guinea pigs has shown that "diagnosis" by cultivation and by fluorescence of acid-stable bound auramine O were almost equivalent (1268). One is led to speculate that the structure of the diphenyl-containing auramine O is better suited for penetrating and stacking along the receptor macromolecules of the mycobacterial peptidolipid and/or peptidoglycolipid than is fuchsin. Aurmaine O also penetrates biological materials very well. The relative fluorescence of auramine O bound, for example, to DNA depends upon concentration of the polymer (899). The intracellular concentration possible with auramine O may be greater than with fuchsin and this may account, in part, for its reputed superiority as a stain for acid-fast mycobacteria.

Acid-stable Binding and Gram staining of mycobacteria. The gram-positive state is equated with the capacity to retain a complex of crystal violet and iodine. It has long been held that "mycobacteria cannot be classified as gram positive or gram negative by the Gram staining technique because, once they have been stained by basic dyes, they cannot be decolorized by alcohol regardless of whether or not they have been treated with iodine" (816, p. 491). This observation stems from the capacity of mycobacterial walls to bind crystal violet in an acid-stable state. However, if one removes the lipoidal portion of the mycobacterial wall with alkaline ethanol (1% KOH in absolute alcohol, wt/vol), there is left an intact bacterial cell which is non-acid-fast but gram positive (L. Barksdale, K.-S. Kim, and C. A. Fisher, unpublished data) i.e., retains the crystal violet-iodine complex. Thus, the apparent "gram-positiveness of tubercle bacilli [mycobacteria] is independent of the mordant effect of iodine and appears to depend upon the same factors which are responsible for acid-fastness" (1064, p. 510). Removal of the structures responsible for acid-fastness enables one to establish the inherent gram-positivity of mycobacteria.

Non-acid-fast mycobacteria. The stages of growth of mycobacteria may be associated with an apparent lack of acid-fastness. It may be absent in a large proportion of actively growing populations of mycobacteria, and it may not be demonstrable in so-called chromophobic phenotypes, which withstand the penetration of fuchsin and other dyes (891). For example, in our hands, actively growing cells of strain 607 are acid-fast only when exposed to hot carbol fuchsin, and then no more than 10% of the cells show good retention of the dye. Cells from Lowenstein-Jensen slants show a similar lack of acid-fastness. Under the discussion of phosphatidylinositol oligomannosides and the mycobacterial cell wall, we point out that pyridine-extracted cells (Fig. 8A and 9) contain tunnels to the cytoplasmic membrane, tunnels once occupied by pyridine-extracted lipids. Further, pyridine extraction greatly modifies the organization of the cell surface and reveals some ringlike structures (Fig. 7C). Such pyridine-extracted cells can be shown to take up fuchsin much more readily than the unextracted cell and, although they are riddled, they retain their acid-fastness. In other words, the normal cell (in this case, *Mycobacterium smegmatis*) is relatively chromophobic. Pyridine extraction lowers the resistance to the penetration of fuchsin without any negative effect upon acid-fast-


A
Mur
PL
Pyr
N
B
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ness. In fact, the enhanced uptake by pyridine-extracted cells results in a higher count of acid-fast bacteria than is found in unextracted samples from the same population of mycobacteria.

Nyka (889, 890) has recommended the use of oxidants for demonstrating chromophobic acid-fast bacilli in tissues. The products of this technique, although useful for showing up additional bacilli, should not be regarded as examples of a mycobacterial acid-fast stain. As pointed out by Fisher (377) even *Escherichia coli* can become "acid-fast" under such conditions. Fisher noted that of the arylmethane dyes only carbol fuchsin, acidified basic fuch-

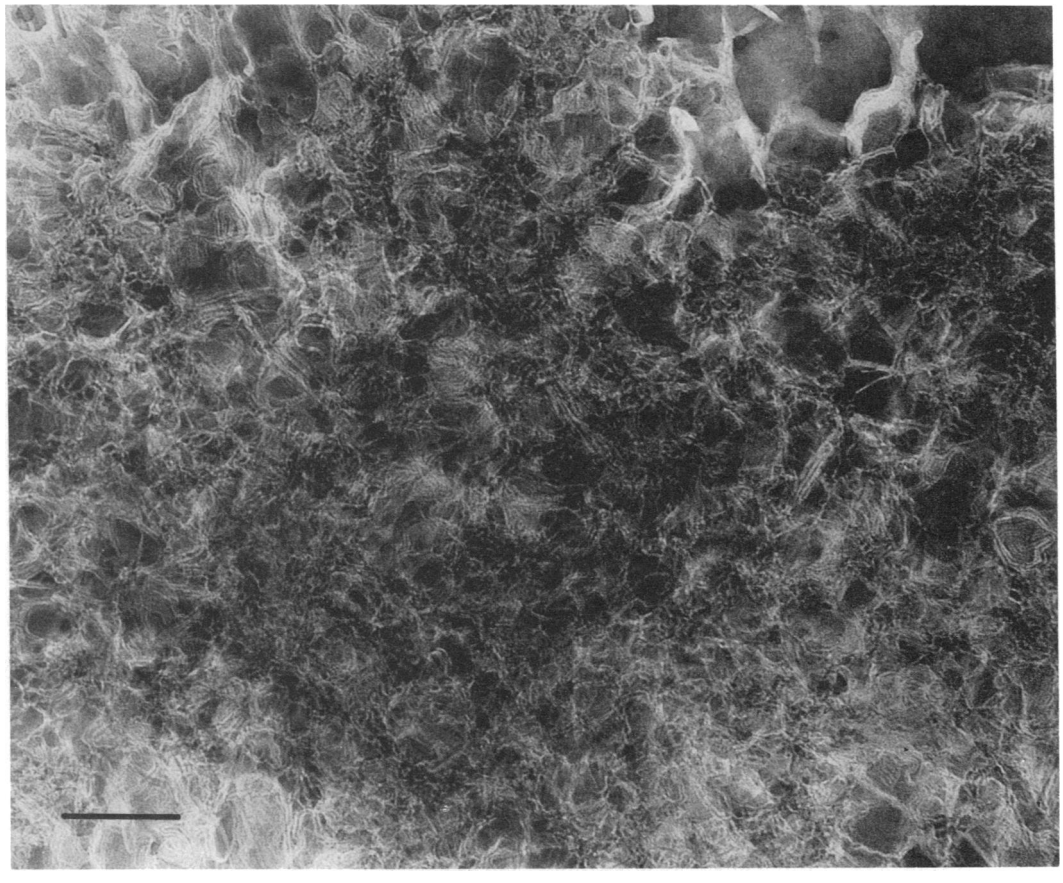


FIG. 9. *Pyridine-extracted material from M. smegmatis 607. Cells were treated as in Fig. 8A. The supernatant pyridine was evaporated to dryness in a Rotavapor and negatively stained. Note laminated structures consisting of fibrils of about 8 nm in diameter, suggestive of ramified fibrous structures seen in mycobacterial cell walls. The removal of this material resulted in the changes shown in Fig. 7C. Note that the extracted cells retained their acid-fastness. (×78,000; bar = 0.2 μm.) For discussion, see section on Phosphatidylinositol Mannosides. See Tables 1, 2, and 3.*

FIG. 8. *Alterations in cell wall profiles of pyridine-extracted (A) and KOH-ethanol-treated (B) cells of M. smegmatis, 607. (A) Cells were extracted with pyridine while shaking for 15 h, 35°C. Note that the peptidoglycolipid layer and the murein (Mur = peptidoglycan) are intact. Here and there are openings (Pyr) created by removal of pyridine-soluble components (see text). The double thickness of the peptidoglycolipid (PL), about 26 nm in width, is evident between two adjacent cells. The remarkable aggregation of these cells suggests that the PL layer has become sticky. Leakage from the cytoplasm is indicated by an absence of the cytoplasmic membrane and mesosomes. The integrity of the ribosomes has been lost. These cells, despite the changes noted, remain acid-fast. (×78,000; bar = 0.5 μm.) See sections on Acid-Fastness and Phospholipids. (B) Section of cells of M. smegmatis 607 shaken in KOH-ethanol (0.5%, wt/vol) for 15 h. Note numerous fibrous structures extending from the cytoplasm and the murein layer. There is no observable cytoplasmic membrane. The peptidoglycolipid layer has been removed. The cells show no tendency to aggregate. They are non-acid-fast. See section, Acid-Fastness. (×78,000; bar = 0.5 μm.) See Tables 1, 2, and 3.*

sin, or aqueous pararosaniline yielded an acid-stable complex with the oxidized bacteria. He attributed this chemical conversion to acid-fastness to the formation of Schiff bases between basic dyes (primary amines) and aldehydes generated by the periodate oxidation of cell wall and membrane components. Kiyoshi Harada has taken the Nyka procedure much further and recommended an acid-fast stain employing an oxidation step involving potassium permanganate (490; see also reference 586). As he puts it, "oxidants which attack ethylenes to form aldehydes were effective in increasing acid-fastness of mycobacteria." He further pointed out that the oxidized mycobacteria reacted so well with methylene blue as to have their newly acquired acid-stable fuchsinophilia obscured. It should be clear from the foregoing discussion that acid-stable fuchsinophilia, acid-fastness of Schiff bases, is not related to *mycobacterial acid-fastness*.

Conditions of Growth

For the most part, mycobacteria have simple growth requirements as compared, for example, with *C. diphtheriae*. Throughout the CMN group, the content of the medium greatly affects the overall syntheses that take place. In the presence of glucose, for example, the major soluble lipids made by *M. smegmatis* and *C. diphtheriae* are acylglucoses in which the hexose is esterified in the 6 position. The major fatty acid (FA) of these acylglucoses is a $C_{15}H_{31}—\underset{\underset{OH}{|}}{CH}—\underset{\underset{C_{14}H_{29}}{|}}{CH}—COOH$ corynomycolic acid.

In the absence of glucose, acylglucoses fail to be made by either of these representatives of the CMN group (183). Acylglucoses may play a role in glucose transport (1049). Quite early, Frouin (409) and Long and Finner (732) found that the composition of the medium in which *M. tuberculosis*, *M. bovis*, and *M. avium* are grown markedly affects the quantitative lipid composition of these organisms. The latter authors found that organisms grown with 12.5% glycerol were much more intensely acid-fast than those grown with 0.5% glycerol. Glucose has been included in media for mycobacteria. In the CMN group, glucose, however, is not always a preferred carbon source. For example, *C. diphtheriae* gives greater yields on maltose than glucose (96, p. 395). The utilization of glycerol by *M. tuberculosis* is markedly inhibited by glucose, and the inhibition is by some mechanism other than catabolite repression. Glutamate is a preferred nutrient for initiation of growth by this *Mycobacterium* as compared, for example, with the traditionally employed asparagine. In a medium containing glycerol and glutamate, there is preferential prior consumption of glutamate. Citrate, commonly employed in mycobacterial media, is not utilized by *M. tuberculosis* $H_{37}Rv$. The apparent growth-enhancing effects of citrate are undoubtedly, as suggested by Dubos and Middlebrook, attributable to the capacity of citrate to chelate calcium, iron, and magnesium, thus preventing their precipitation from liquid media (322). Shaking is a preferred method of cultivation, and small inocula are to be avoided (162). Carbon dioxide, 6% (872) to 8% (476), enhances growth and is essential for growth from certain small inocula. Antoine and Tepper (47) have demonstrated an effect of limitation of nitrogen or sulfur on accumulation of glycogen and lipid in *M. phlei*. Tanaka and associates (1139) have reported an effect of nutrient (*n*-alkanes versus glucose) on the fragility of cells of *M. smegmatis*. Their electron micrographs revealed that the more fragile hydrocarbon-grown cells were round and possessed a comparatively ill-defined cell membrane. David has shown that when a population of $H_{37}Rv$ is subjected to a nutritional shift-down by transfer from a medium supplemented with amino acids to a basal medium (liquid or solid), a large number of cells fail to survive the transfer. Similar disparities in number of surviving *M. tuberculosis* are obtained when inocula from in vivo sources are plated out on Middlebrook 7H10 agar and the more enriched Middlebrook 7H11 agar. The failure to survive has been ascribed to an inability of the cells to adapt from a state of end product repression (285). Other examples of effects of environment on mycobacterial biosyntheses are scattered throughout this paper.

We have previously pointed out the remarkable capacity of certain members of the CMN group to be stimulated by pyruvic acid (96). In 1967, Dixon and Cuthbert pointed out that recovery of *M. tuberculosis* from sputa was markedly enhanced in egg media in which glycerol had been replaced by pyruvate. Earlier observations on the enhancement of mycobacterial growth by pyruvate are alluded to by these authors (306).

The Mycobacterial Nucleoid

The DNA of the nucleoid of $H_{37}Rv$ (Fig. 1) occurs in association with a lamellated concentration of some of the cytoplasmic membrane system, a mesosome. The size of the genome of this particular *Mycobacterium* has been estimated to be 2.5×10^9 daltons. This is approximately the same amount of nuclear DNA as is found in *M. avium* (2.9×10^9), *M. bovis* BCG

(2.8×10^9), and *M. intracellulare* (2.5×10^9), but less than that comprising the genomes of *M. kansasii* (3.8×10^9), *M. marinum* (3.9×10^9), and *M. smegmatis* 405 (4.5×10^9) (175).

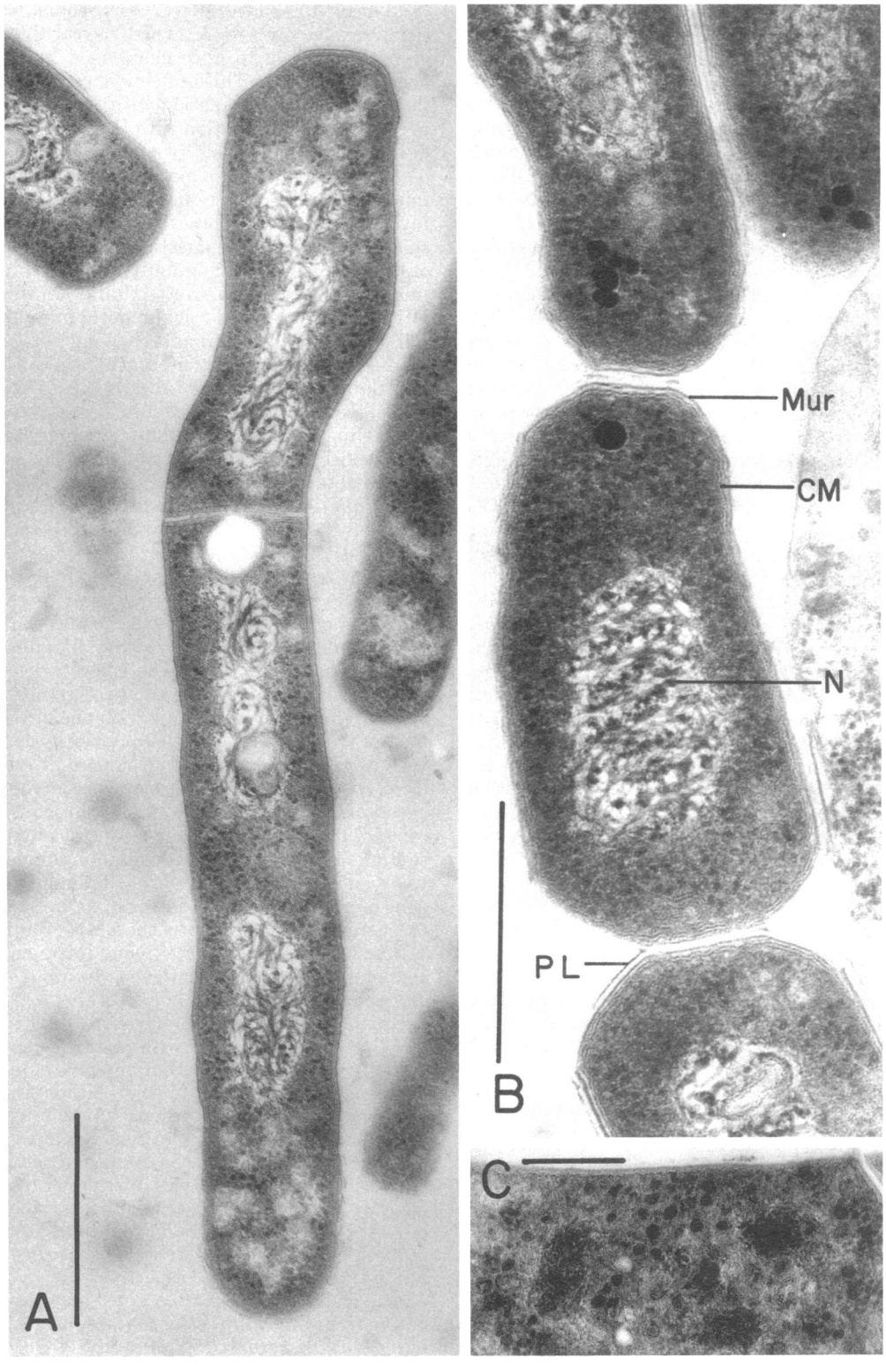
The guanine plus cytosine (G+C) contents of mycobacteria have been examined by a number of workers (384, 483, 1061, 1140). Randomly chosen percent values for G+C from Wayne and Gross follow: *M. tuberculosis* $H_{37}Rv$, 65.0; *M. kansasii*, 65.7; *M. marinum*, 65.0; *M. xenopi*, 65.5; *M. fortuitum*, 65.1; *M. flavescens*, 65.4; *M. avium*, 68.5; *M. intracellulare*, 67.3; *M. phlei*, 67.4 (1233). Optical measurements of reassociation of various mycobacterial DNAs with reference DNA from *M. bovis* BCG and *M. farcinica* 436 have been carried out by Bradley (175; see also reference 176). Puzzling among these findings was a zero reassociation between *M. marinum* and both BCG and strain 436 DNAs (175) under conditions where DNAs from *M. phlei* and *M. smegmatis* 461 gave 23 and 22% reassociation, respectively, with BCG. By this same method, the percent "optical reassociation homology" between *M. marinum* DNA and DNA from *M. smegmatis* 405 gave a value of 19% when DNAs from *M. bovis* BCG, *M. intracellulare*, *M. fortuitum*, and *M. phlei* were, respectively, 12, 26, 37, and 45%. Bradley has pointed out the limitations of such qualitative studies (176). Although DNA is the genetic heart of the matter, there are problems that limit the value of hybridization studies for taxonomic purposes. The difficulties partly rest with problems in purifying DNA preparations from certain species. Hill et al. have discussed the matter of the contamination of DNA from BCG, presumably with arabinogalactan. They found the association between polysaccharide and DNA to be tight and to exert a real effect on the accuracy of optical determinations of G+C content. However, the presence of the polysaccharide did not adversely affect thermal denaturation values (513). Mizuguchi and Tokunaga (839) have described a gentle method for obtaining DNA from *M. smegmatis* 607 that uses glycine and lysozyme.

Mycobacterial Ribosomes

The ribosomes of $H_{37}Rv$, shown in Fig. 1 (see also Fig. 10 for *M. smegmatis* 607), have been studied by Worcel et al. as 70S units and have been separated into their component 50S and 30S subunits (1282). Youmans and Youmans have pioneered in the use of mycobacterial ribosomes for immunity studies (1300, 1301). These authors have reviewed sedimentation properties of ribosomal ribonucleic acid (RNA) from $H_{37}Ra$ in relation to immunogenicity and adjuvant action (1302). Recently, Neiburger et al. (873) have studied the effects of ribosomal fraction, termed myc RNA, which is capable of giving protection to mice challenged intravenously with $H_{37}Rv$. This protection was equal to that afforded by vaccination with $H_{37}Ra$. However, whereas vaccination with $H_{37}Ra$ led to the development of delayed hypersensitivity (DH) as measured by specific macrophage migration inhibition (MIF) techniques, vaccination with myc RNA did not. These findings have been said to again raise the question of the meaning of DH to tuberculin in immunity to tuberculous infection and recall the work of Raffel, in which DH could be separated from humoral immunity (HI), and only those animals showing DH gave a "modified Koch reaction" to intracutaneous inoculation with virulent tubercle bacilli (953), as well as the work of Fong et al., which shows that cellular resistance ("immune monocytes") could exist in the absence of DH (385). (For related details, see section DH and HI in *Mycobacterium* as Antigen and Tuberculin Anergy.)

Baker et al. (87), using immunoelectrophoretic methods, have begun an examination of relatedness of ribosomal antigens from BCG and $H_{37}Rv$ ribosomes and the relatedness of both sets of ribosomal antigens to those from cytoplasmic fractions and culture filtrates of the two bacterial species. The ribosomal antigen-antibody systems from the two mycobacteria shared many interactants with one another but few with systems of antigen-antibody developed from cytoplasm and from culture filtrates. Here, as with all comparative assessments based on immunodiffusion, well-characterized reference antigens and their antibodies are essential. Once these become available, comparative evaluations can be made of ribosomal antigens of a variety of mycobacteria, as well as of those from other members of the CMN group.

In mouse protection tests (using intravenous challenge) ribosomal antigens from *Histoplasma capsulatum* (plus adjuvant) have given adequate protection. This suggests that ribosomal vaccines, effective within such a mouse system, can be prepared from any of a number of disease-producing organisms. Although the exact molecular configurations functioning as antigen in ribosomal preparations remain to be described, there seem to be certain structures that cannot be violated without rendering ineffective the ribosomal antigen. Thus, ribonuclease (RNase), trypsin, and Pronase treatment reduced effectiveness in the case of *H. capsulatum* by 85, 50, and 55%, respectively (364). The effectiveness of ribosomal vaccines may simply derive from their content of contaminating immunogens. A recent critical study of *Salmonella* ribosomal vaccines indicated that, indeed,


A
Mur
CM
N
PL
B
C

contaminating O antigens were responsible for the effectiveness of the so-called *ribosomal* vaccine (336).

Rifampin, DNA-directed RNA polymerase and the breakdown of ribosomes. Rifampin, a semisynthetic antibiotic derived from rifamycin B (a fermentation product of *Streptomyces mediterranei*) affects bacteria and other susceptible organisms by specifically blocking DNA-dependent RNA polymerase (see review by Wehrli and Staehelin [1237]). Similarly, DNA-dependent RNA polymerase isolated from a rifampin-sensitive strain of *M. smegmatis* (1253) and of *M. bovis* BCG were blocked by rifampin (830). Although 1 μg/ml blocked the activity of the polymerase from sensitive *M. smegmatis*, enzyme from a resistant mutant was unaffected. At least two forms of resistance appear to occur: (i) those involving resistant enzyme and (ii) those in which uptake of the antibiotic is reduced or inhibited (830). The overall effect of rifampin on a susceptible cell is one of total breakdown, as shown by Konno et al. (642). These investigators have published a series of ultrathin sections of $H_{37}Ra$ subjected to 10 μg of rifampin per ml for periods of 6, 12, 24, and 48 h. Breakdown of cellular organization was evident at 12 h: e.g., although the nucleus, the cytoplasmic membrane, and the cell wall remained intact, ribosomes disappeared, the cytoplasm became vacuolated, and mesosomal structure seemed affected. Thus, the more obvious effects were those associated with messenger RNA, protein, and ribosomal biosyntheses.

Genetics of Mycobacteria (Origins of $H_{37}Rv$)

Petroff and Steenken (924), in reviewing the literature of "microbic dissociation" up to 1930, made it clear that nonrecombinational genetics among mycobacteria, i.e., genetic variation or mutation, followed a pattern already established for a number of bacterial species. The selection of the bile-tolerant mutant of *M. bovis* BCG had been established 22 years earlier (213). Present-day descendants of *M. tuberculosis* strain H_{37} (isolated by E. R. Baldwin in 1905 from the sputum of a patient, age 19, suffering from chronic pulmonary tuberculosis), the mutants $H_{37}Rv$ and $H_{37}Ra$, were isolated, characterized, and reported in two papers by Steenken et al. (1092, 1093). Steenken stated, "for clarity it seems advisable to discontinue this usage of 'R' and 'S' and to employ them in the usual sense as indicative of rough and smooth colony structure. To indicate virulence or avirulence the letters (v) or (a) are appended thus 'Rv' and 'Ra'. Since a smooth variant of H_{37} which manifests a typical morphology and virulence has not been obtained, this terminology has been adopted to cover the virulent and avirulent strains having an 'R' morphology" (1092). What is meant here is that R will no longer stand for Resistant to an inimical environment (Gentian violet-egg medium, pH 6.1), and S will no longer stand for Sensitive to an inimical environment. By making the change, these authors also avoided confusion with the R and S of the pneumococci and certain other microorganisms where smoothness *is* associated with virulence. Petroff and Steenken established the fact that old cultures of *M. tuberculosis* accumulate mutants (Resistant to pH 6.1, bile, etc., and Sensitive to the same), and the Resistant mutants may be selected on those media which do not favor the growth of Sensitive strains. Their continued cultivation on the selective media tends to suppress the development of populations of the sensitive mutants. Other conditions were described which favored the outgrowth of the *sensitive* but not that of the *resistant* strains. The association of distinctive colonial morphology and degrees of virulence with Rv and Ra was indeed fortuitous. Today, drug-resistant strains of mycobacteria are not uncommon and, in time, drug resistance markers will be useful in mycobacterial genetics. Undoubtedly plasmids such as resistance transfer factors occur in mycobacteria and probably often are related to the drug resistance patterns found in various species. To date, however, there is only the most circumstantial evidence for their existence (563). A

FIG. 10. *(A) Section of M. smegmatis 607 cells showing a mesosome-like structure (minus extracted internal substances) adjacent to newly completed cross wall. Membrane-associated lipid inclusions are frequently observed in actively growing cells. In "aging" cells larger, vacuole-like inclusions are common. (×65,000; bar = 0.5 μm.) (B) Section of actively growing cells of M. smegmatis 607 showing nucleus in center and polysomes in cytoplasm. An electron-transparent peptidoglycolipid layer (PL, about 13 nm in width) is located external to the murein layer (Mur). Several granules (probably polyphosphate) appear in this micrograph. Their sizes vary from 25 nm to 65 in diameter. (×78,000; bar = 0.5 μm.) (C) Section of Mycobacterium sp., strain NQ showing many densely stained inclusions (average 30 nm in diameter) in the cytoplasm. For some, only a limiting peripheral vesicle remains. (×75,000; bar = 0.2 μm.) In text (A), (B), and (C) are discussed under The Mycobacterial Cell, Biosynthesis of Lipids, and Murein-Arabinogalactan. See Tables 1, 2, and 3.*

search for such plasmids will probably be very fruitful.

It has been well established that ultraviolet UV irradiation (640, 837), ethyl methane sulfonate (641), nitrous acid (952), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and phenethyl alcohol (835, 1312) are suitable mutagens for mycobacteria. At least two transducing mycobacteriophages have been reported (425, 426, 658, 955, 956). One of these is illustrated in Fig. 31C and D. It is remarkable for a CMN phage, for it has an elaborate tail apparatus suggestive of the coliphages T2, T4, and T6 (42) and belongs to Bradley's group A (174). The existence of suitable mutagens and a pair of transducing phages augurs well for progress in the genetics of mycobacteria. Genetic recombination has already been demonstrated to occur between *M. smegmatis* and *M. smegmatis* (*lacticola*) (840) where the donor bacterium, *M. smegmatis* (*lacticola*) (for synonymy, see Table 2), provided only a few genes (836) in the formation of the recombinant merozygotes (542). This is suggestive of recombination in *E. coli* (501) and *Streptomyces coelicolor* (520), and between *Nocardia canicruria* and *N. erythropolis* (193). In this review are listed many well-established genetic markers of mycobacteria. It would seem that the markers and the methodologies needed in studies of mycobacterial genetics are at hand, and that all that is required now is a search for new mating pairs, interest in the subject, and the use of a modicum of caution. For example, Hartwigk and Hummel (498), using single-cell isolation techniques, point up the fact that sometimes cultures of mycobacteria are mixed. They found the Nassal strain 396 of *M. tuberculosis* to be a mixed culture of *M. tuberculosis* and *M. avium*.

The future of mycobacterial genetics seems assured, and the genetics of control systems governing the biosynthesis of mycolic acids, the assemblage of mycobacterial cardiolipins (CLs), arabinogalactans, arabinomannans, phosphatidylinositol oligomannosides, etc., offers exciting territory for tomorrow's molecular genetics.

Autolysis, Protoplasts, and Mycobacterial L-Forms

Old cultures of mycobacteria often undergo considerable autolysis, the initiation of which can result from any of several conditions leading to unbalanced metabolism. The depletion of the nitrogen supply in the presence of an ample carbon source (1027) and severe oxygen limitation in the presence of ample triose (1229) have been studied as conditions that bring on autolysis of mycobacterial cultures. Cultures in which autolysis has been induced by oxygen limitation, upon adequate oxygenation, yield a burst of new growth (1229). Thus, *there are a number of viable units that survive in preponderantly autolyzed cultures*. Old broth cultures (and some old slants) of mycobacteria are sources of mycobacterial cells of coccal shapes and altered staining properties (1167, p. 541; 270, 1096). Csillag (270) has shown that *clones* of *coccal forms*, derived by streaking on nutrient agar from aged broth cultures of a streptomycin-resistant strain of *M. phlei, themselves* carry the streptomycin-resistant marker. This and other experiments of Csillag are very important for understanding cell form variants reported in mycobacteria from the time of Much (1167, p. 541). In this connection, see Imaeda and Rieber (532) and Krassilnikov (661, 662).

Clasener (253) has reviewed the pathogenicity of L-phases of a number of bacteria and concluded that L-phases per se are probably incapable of establishing an infection. Much has been written about "wall-less mycobacteria" and their possible role in infections. A recent paper by Korsak (646) reviews the subject and points up the problems in assessing the importance of such forms. The presence of proven mycobacterial L-forms certainly indicates the presence of a mycobacterial genome for which there is a reasonable chance of mutation to the infectious walled organism. See section, Mycobacteria Growing In Vivo and In Vitro.

With regard to L-forms and granulomas, Spector has reported an intriguing observation: "whereas an A strain hemolytic streptococcus causes, on injection, acute inflammation followed by fibrosis, the L-form variant of the same organism causes a persistent granuloma" (1078).

LIPID BIOSYNTHESIS

Fatty Acids in General

Two general systems for the biosynthesis of FAs have been discovered during the last two decades (178, 743, 1194, 1213): (i) a stable multienzyme complex effecting at least seven specific enzymatic reactions is found in mammals, birds, and yeasts. Each reaction in the yeast system involves the covalent bonding of the substrate and intermediates to the FA synthetase complex (744; see also [66]). (ii) The second "system" involves enzymes associated with the soluble fraction from such cells as *E. coli*. These, in the presence of 4'-phosphopantotheine acyl carrier protein (ACP), carry out FA biosynthesis (410, 764, 1211). In both systems,

the thiol that is esterified to the growing fatty acyl chain is a part of 4'-phosphopantotheine-containing ACP. The overall process involves (i) initiation with CH_3—CO—SCoA to yield (ii) COOH
|
CH_2—CO—SCoA + CH_3—$[CH_2—CH_2]_n$—CO—S—ACP and elongation through to (iii) CH_3—$[CH_2—CH_2]_{n+1}$—CO—SCoA and termination. Nicotinamide adenine dinucleotide phosphate ($NADP^+$) and flavin mononucleotide (FMN) are involved in the middle steps of elongation. Konrad Bloch and his associates have shown that *M. phlei* possesses a high-molecular-weight (1.39×10^6) multienzyme FA synthetase (1198), originally named FA synthetase type I (187) (FAS I) and distinguishable from the type II synthetases (nonaggregated), FAS II, which require for their activity the *addition* of protein-bound 4'-phosphopantotheine ACP and the presence of each of the readily separable catalysts in the multistep pathway. Employing a system (FAS I of *M. phlei* plus acetyl-coenzyme A [CoA] and malonyl-CoA) yielding triacetic acid lactone in the presence of either NAD^+ or $NADP^+$, White et al. showed that $NADP^+$ was the specific coenzyme for the β-ketoacyl reductase step, whereas NAD^+ was preferentially utilized in α-β-enoyl reduction (1250).

In the *M. phlei* FAS I system, the presence of FMN and of mycobacterial polysaccharides containing either 3-*O*-methylmannose (MMP) or (lipo)polysaccharides containing 6-*O*-methylglucose results in maximal synthesis (see Fig. 11 and reference 529). CoA and CoA derivatives of C_{16}, C_{18}, C_{20}, and C_{22} FAs form tight complexes with these polysaccharides, and the complexes travel together on Sephadex columns. In the case of the longer-chain FA, the complexes were composed of equal molar amounts of acyl-CoA derivatives and polysaccharide (750). Alpha-, beta-, and gamma-cyclodextrins, in that order of effectiveness, were also found to stimulate (as 6-*O*-methylglucose- and MMP-containing polysaccharides) FAS I of *M. phlei*. 2,6-Di-*O*-methyl-α- and -β-dextrins were several times more stimulatory (749). The classes of FA produced by FAS I of *M. phlei* are of two groups, one of shorter chains (C_{14} to C_{18}) and one of longer chains (C_{20} to C_{24}). The relative proportions of palmitate and tetracosanoate, on the one hand, to myristate, stearate, arachidonate, and behenate, on the other, can be varied by: (i) raising the acetyl-CoA to malonyl-CoA ratio, (ii) the addition of one of the stimulatory *O*-methylhexose-containing polysaccharides, or (iii) the addition of bovine serum albumin (BSA). Each of these changes or additions

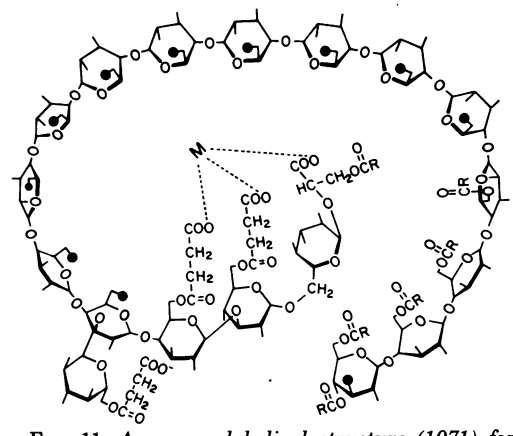


FIG. 11. *A proposed helical structure (1071) for the 3-O-methyl-D-glucose-containing lipopolysaccharide [predominantly α-(1 → 4)-linked] of M. phlei (MGLP-IV) containing 10 molecules of 6-O-methyl-D-glucose, 1 molecule of 3-O-methyl-D-glucose, and 7 molecules of D-glucose. It is acylated by acetyl, propionyl, isobutyryl, octanoyl, and succinyl groups and is linked to D-glyceric acid. It thus has a hydrophilic side where are concentrated most of the hydroxyl groups and a hydrophobic side where acyl groups and methyl groups (●) predominate. It has been suggested that the dual nature of MGLP (hydrophilic/hydrophobic) suits it to function at the interface between membranes and cytosol. MGLP has been shown to be capable of activating FAS I (see text and [529]). Further, the juxtaposed carboxyl groups of D-glyceric acid and one of the two monoesters of succinate (or the two adjacent succinate carboxyl groups) could allow for metal ion (M) chelation. In artificially prepared membranes, chelation of Ca^{2+} by MGLP has been demonstrated (607). See Tables 1, 2, 3. Reprinted with permission of the authors and publishers (470).*

brings about a shift in favor of shorter-chain acids (381). [Lee and Ballou (708) first noted the 6-*O*-methylglucose-containing polysaccharides from *M. tuberculosis* $H_{37}Rv$ and *M. phlei*, and these were defined by Lee (707) and further characterized in reports by Saier and Ballou (1006–1008). Ferguson is reported to have isolated methylglucose-containing lipopolysaccharides (MGLP) from the cytoplasm of sonically disrupted *M. phlei* (607). An intracellular localization for the 6-*O*-methyl-D-glucose-containing soluble polysaccharide of *M. tuberculosis* $H_{37}Ra$, which differed slightly from the polysaccharide described by Lee, has been suggested by Lornitzo and Goldman (735). Keller and Ballou (607) have resolved a 6-*O*-methylglucose-containing lipopolysaccharide from *M. phlei* (see also references 467, 1006–1008) into four components with zero to three monoesterified succinate residues. Each component contained four additional acyl groups: acetate, propio-

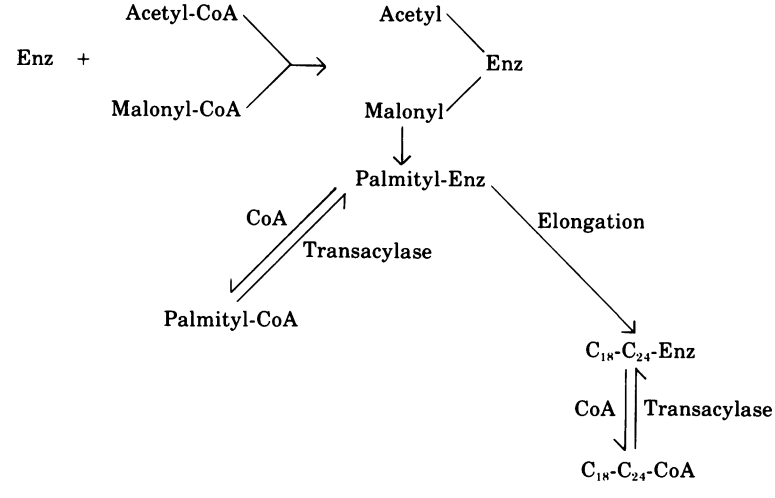
nate, isobutyrate, and octanoate, postulated to be present in molar ratios of 3:1:1:1 (Fig. 11). As yet, no linkage has been established between the 6-*O*-methylhexose-containing polysaccharides with an affinity for FAS I and the 6-*O*-methylglucose-containing *lipo*polysaccharides of various mycobacteria.]

Allen et al. have reported the isolation from *M. smegmatis* of a propionyl-CoA carboxylase (containing tritium-labeled biotin) whose activity was stimulated by glutathione (23). It is not known whether this carboxylase is separable from the acetyl-CoA carboxylase activity of *M. smegmatis*. Erfle has described an enzyme from *M. phlei* that carboxylates both acetyl-CoA and propionyl-CoA. The enzyme is an aggregate enzyme complex. No similar activity of a disaggregated complex was found (350).

The discovery of an FAS I in *C. diphtheriae* seems to have come as a surprise to Knoche and Koths (622), who consider it a primitive organism. As a member of the CMN group, having much in common with *Mycobacterium* and *Nocardia*, it is no surprise to learn that the corynebacterial FAS I is similar to FAS I of *M. phlei*: e.g., it is stimulated by NAD^+ and $NADP^+$, is stabilized by 0.5 M phosphate buffer and falls apart in buffers of low ionic strength (it is irreversibly inactivated in 0.01 M phosphate buffer at 37°C for 5 min), and is stimulated by BSA (622), as well as a particular succinate-containing MGLP (621). The corynebacterial enzyme is inhibited by longer-chain acyl thioesters (C_{16} to C_{20}) and is protected from this inhibition by BSA. Thus, the stimulatory effect is actually a release from inhibition. Identical relief from acyl-CoA inhibition is provided by MGLP from *M. phlei*. Complexing of either BSA or MGLP with palmityl-CoA results in an increase in the overall rate of synthesis from acetyl-CoA and malonyl-CoA and a shift in the pattern of FA synthesis towards products of shorter chain lengths.

Recently, Flick and Bloch have described an *M. phlei* palmitoyl thioesterase which, by thioester hydrolysis, may lower the levels of free or enzyme-bound C_{16} CoA (or C_{18} CoA) and therefore affect the rate of synthesis, the sorting of products, or both. Thus, there are three effects operating to alter chain lengths in the *M. phlei* FAS I system: (i) shifting the ratio of acetyl-CoA to malonyl-CoA, (ii) adding either MMP, MGLP, or BSA to the system, or (iii) adding palmityl thioesterase (381).

In summary: FAS I activity is highest with acetyl-CoA. The following reaction scheme adapted from Vance et al. (1197) indicates that the FAS I system of *M. phlei* leads both to FA synthesis (on the left) and to FA elongation (on the right). When the system is tested for de novo synthesis (left side of diagram), fatty acyl-



CoA derivatives from C_{14} to C_{26} are formed (187). The major products are palmitate and tetracosanoate (1197). The most efficient primer is acetyl-CoA, with efficiencies dropping to about one-tenth, one-twentieth, and one-one-hundredth with propionyl-CoA, butyryl-CoA, and hexanoyl-CoA, in that order. The addition of polysaccharide (MMP) markedly enhances yields for each of these FA-CoA's, being about 9-fold in the case of acetyl-CoA, 30-fold for hexanoyl-CoA, and 20-fold for both butyryl-CoA and propionyl-CoA (1198). Chain elongation (right side of diagram) occurs with acyl-CoA derivatives of carbon numbers up to C_{16}, and the end products are principally stearate and

tetracosanoate. When purified FAS I is used, MMP or MGLP is required for chain elongation. Longer chains (elongation of decanoyl-CoA, for example, are not attained with much efficiency unless there is added an elongation factor, a partially purified protein associated with the long-chain acyl-CoA thioesterase. Apparently, elongation factor (inseparable from long-chain acyl-CoA thioesterase) releases free CoA from substrates (1197).

When Brindley et al. first reported FAS I from *M. phlei*, they also reported the presence of free ACP in this organism, and for this presence they offered two explanations: (i) that the multienzyme complex dissociates in vitro, releasing free ACP, or (ii) that the two FASs (I and II) operate separately in *M. phlei*. Indeed, they did find a second system, of average molecular weight less than 250,000, which was dependent on added ACP and used palmitoyl-CoA or stearoyl-CoA, but neither octanoyl-CoA nor acetyl-CoA, for chain initiation (187). $ACP_{M.\ phlei}$ was subsequently found to have a molecular weight of 10,600 and an amino acid composition slightly different from that of $ACP_{E.\ coli}$ (796): four prolines (one in $ACP_{E.\ coli}$) and no histidine (one in $ACP_{E.\ coli}$). $ACP_{M.\ phlei}$ supports malonyl-CoA-CO_2 exchange in *E. coli* extracts with about half the activity found with homologous $ACP_{E.\ coli}$. It is unable to replace $ACP_{E.\ coli}$ in the *E. coli* FAS II system. It actually inhibits the reaction, despite the fact that in the transformation of stearoyl-CoA to longer-chain FA by *M. phlei* both $ACP_{M.\ phlei}$ and $ACP_{E.\ coli}$ can serve as active acyl carriers (795). This chain-lengthening activity is absolutely dependent on added supply of ACP (796). So far, there has been no report of a FAS I in other mycobacteria, nor has there been additional information on the FAS II of *M. phlei*.

Fourteen years ago, the Kusunoses et al. demonstrated the incorporation of label from [^{14}C]acetate and [^{14}C]malonate into FA in soluble extracts of *M. avium* (677). With added [^{14}C]malonate, most of the label appeared in FA above C_{16}, especially C_{24}. Bicarbonate was an important component of the system. Goldman and associates have examined FA-elongating and -synthesizing soluble extracts from *M. tuberculosis* $H_{37}Ra$ (576, 929, 1219). Recently, Besert and Etémadi (128, 129) have shown that the palmityl-CoA-ACP transacylase (molecular weight, 100,000), the malonyl-CoA-ACP transacylase (molecular weight, 32,000), and the acetyl-CoA-ACP transacylase (molecular weight, 85,000) activities, derived by ammonium sulfate fractionation of 105,000 × *g* supernatants of *M. smegmatis* sonically disrupted (in 0.1 M phosphate buffer, pH 7, containing 0.0001 M ethylenediaminetetraacetic acid [EDTA] and 0.01 M mercaptoethanol), are completely separable. ACP from *E. coli* functioned in this FAS II system. These authors apparently found no evidence for a multienzyme FAS I in *M. smegmatis*. They have discussed in some detail the possible meaning of their findings in terms of an FAS I system, as reported for *M. phlei*, and the FAS II system, which functions in *E. coli*. If *M. phlei* is the archetypal *Mycobacterium* Twort and Ingram have thought it to be (1189), it *may* have far more elaborate machinery for the synthesis of lipids than some of its derived cousins. Since, however, Bloch and associates have found FAS I in *C. diphtheriae*, it would be surprising not to find such activity in mycobacteria other than *M. phlei*.

Diglycerides, Triglycerides, Lipid Globules, Fat Bodies, and Intracellular Storage of Lipid

Burdon (205) was impressed with the inclusions revealed in members of the CMN group by staining with Sudan black B. In Fig. 2 are shown side-by-side sections of a cell of *M. aurum* and a cell of *M. tuberculosis* $H_{37}Rv$ showing areas once occupied by fat bodies. Schaefer and Lewis (1026) have demonstrated that apparently similar inclusions can be induced to appear in cells of *M. kansasii* whose growth has been stimulated with detoxified oleic acid (as oleate-BSA-fraction V complex) or its ester, polyoxyethylene sorbitan mono-oleate. Uptake of oleate was rapid. First the cells became coated with lipid (which modified their optical properties), to which Sudan black B fixed. The intracellular accumulations of lipid appeared under phase microscopy as bright globules. At 4 to 7 h postincubation with lipid, the globules could be seen at the ends of the cells and, at 18 h, their numbers had increased to six or seven per cell. These fatty inclusions corresponded with the Sudan black B-stained inclusions demonstrable in the same cells. Electron micrographs of 19-h-old cells revealed inclusions morphologically similar to those shown in Fig. 2A and B (for initial lipid accumulation associated with cytoplasmic membrane, see Fig. 10A). The authors showed that synthetic triolein, elaidic acid, lecithin, cephalin, polyoxyethelene sorbitan monolaurate, and sodium laurate, as well as high concentrations of human, bovine, rabbit, and guinea pig sera, stimulated the appearance of the globular inclusions. Whereas *M. kansasii* and *M. marinum* responded to both oleic acid and esterified oleate, as Tween 80, *M. avium* formed globules in the presence of oleic acid but not Tween 80. This finding was correlated with the inability of *M. avium* to hydro-

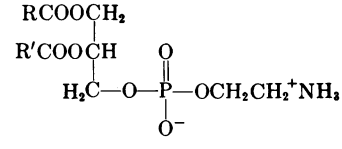
lyze Tween 80. The extent to which the FAs contained in lipid globules are in some way modified was not determined in these experiments. McCarthy (801) has reported pH-sensitive, temperature-dependent uptake of [^{14}C]palmitic acid by *M. avium*. Uptake proceeds with saturation kinetics and, within 5 min after the cells are brought into contact with the FA, the latter is incorporated into triglycerides of the cell. In turn, some of the triglyceride is rapidly utilized, as indicated by the evolution of $^{14}CO_2$. Gas-liquid chromatographic analyses of these triglycerides of *M. avium* indicated the FAs in greatest concentration to be palmitic, palmitoleic, and oleic acids. FAs above C_{18} and below C_{16} were also present. In fully grown *M. avium*, not starved for FA, the level of triglyceride was found to be close to 5% of the dry weight of the cells. For other relationships between *M. avium* and palmitic acid, see McCarthy (802). It has been shown by Antoine and Tepper that nitrogen-limited *M. tuberculosis* undergoing postexponential growth in a glycerol-containing medium accumulated considerable lipid (48). Although this lipid was not identified, McCarthy has suggested that it was triglyceride (801). Early demonstrations of triglycerides in mycobacteria (142, 814) did not localize these compounds in the cell. Their existence in *Mycobacterium* has long been known, and Asselineau has reviewed their distribution among some members of this genus (59, p. 157). Walker and co-workers have reported that the triglycerides of BCG and *M. smegmatis* are esterified mainly with C_{18}-related FA in position 1 (18:0, 18:1, and 19 BR), C_{16} FA in position 2, and C_{20} and higher FA in position 3 (1216). They found a marked difference between the C_{20+} FA at position 3 in the triglycerides of BCG (76%) as compared with *M. smegmatis* (43%). More recently Weir et al. (1239) have studied uptake and incorporation of oleate into triglycerides of *M. smegmatis* in relation to the appearance in the cells of lipoidal bodies. They suggested that early oleate entering the cell ended up in triglycerides and that once the acceptors (diglycerides?) were used up, remaining oleate accumulated as *lipoidal droplets*. Their reasoning was: "the initial rapid incorporation of oleate into triglycerides may be the result of esterification of the small amounts of free diglycerides present. When the endogenous diglyceride is exhausted, the incorporation rate decreases, slower incorporation occurring as diglyceride is made available by metabolism and by lipase hydrolysis of phosphatidic acid and other phospholipids." McCarthy's results indicate that stored triglycerides offer a ready source of energy to the cell, as well as a means of keeping intracellular free FA at nontoxic levels. Her findings, taken with those of Schaefer and Lewis, suggest that a number of different FAs may provide molecules for tri- and phosphoglyceride stockpiles. For example, we have found that cells of *Mycobacterium* sp. 1081, growing in the footpads of mice, at 2 months postinfection show remarkable accumulation of lipoidal bodies (Fig. 12).

Glycosyldiglycerides have been found in nocardias (612), and a cell-free system that forms them from uridine 5'-diphosphate (UDP)-galactose and UDP-glucose has been isolated from *M. smegmatis* (1035), where it has been suggested that they may function as reservoirs and in the transport of hexoses across the membrane, or both (see also [1049]).

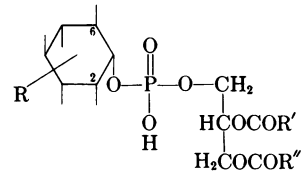
Sometimes, the areas of accumulated lipid are surrounded by polysomes (Fig. 13). Such lipid storage areas appear to be one of the hallmarks of the postexponential mycobacterial cell. We have previously pointed out aspects of lipoidal bodies of other members of the CMN group (96). That the role of triglycerides in storage may be very different from their role in the actively metabolizing cell is suggested by the work of Brennan et al. (184), whose results indicated a high rate of turnover of triglycerides. This suggested to them that triglycerides could not be thought of as metabolically stable storage compounds. They pointed out that there is a high rate of labeling of triglycerides in *Chlorella vulgaris*. What may be important here with regard to triglyceride turnover versus storage is the physiological state of the cells being studied. Throughout this review, there are reported examples of marked differences in biosyntheses in log-phase cells as compared with cells not undergoing exponential growth.

Phospholipids and Glycolipids

The phospholipids of mycobacteria include: phosphatidylethanolamines (PE):



diphosphatidylglycerides (CLs), and the myo-inositol phospholipids (20, 91, 709),



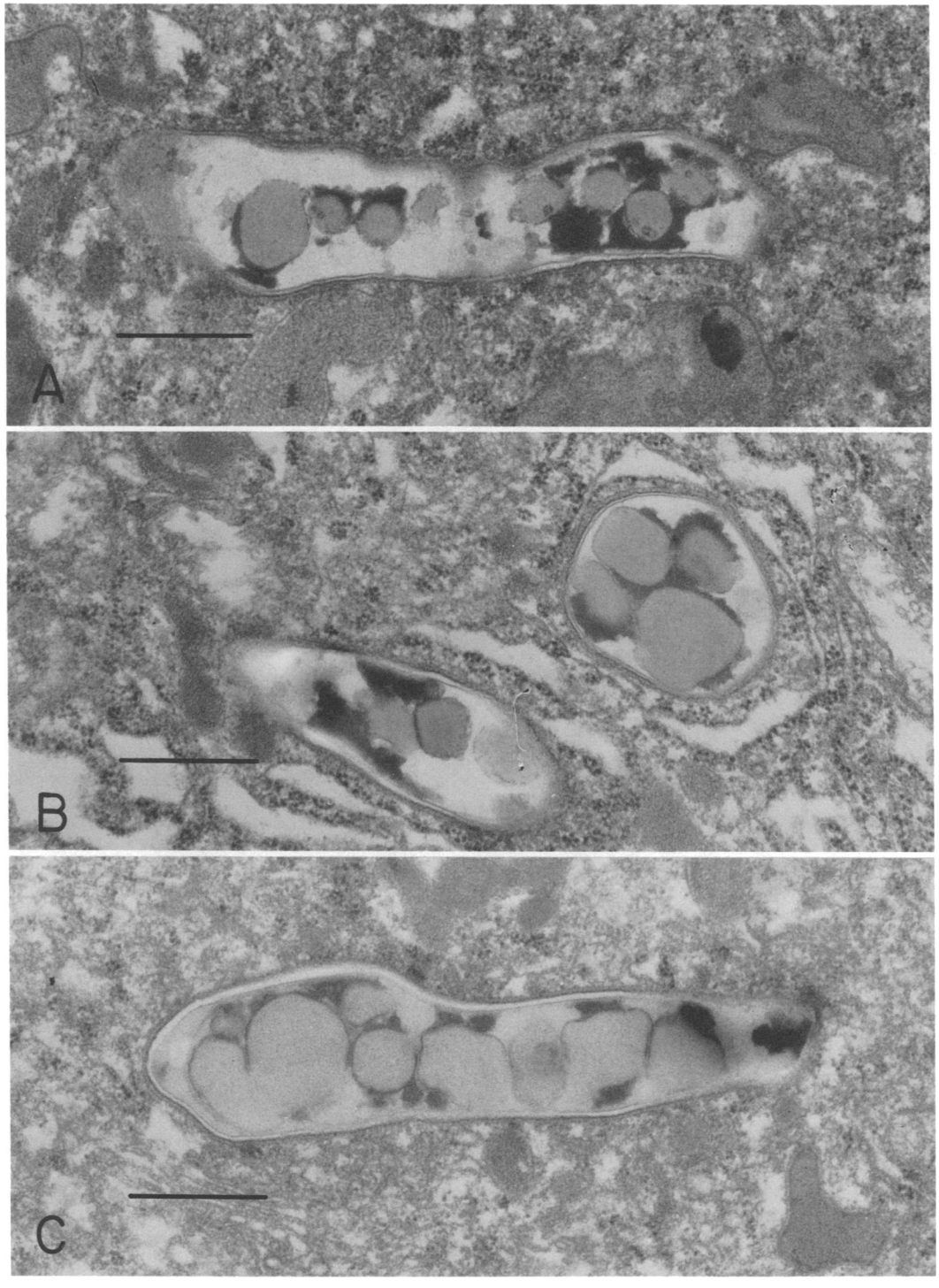


FIG. 12. *Lipoidal inclusions in in vivo-grown Mycobacterium sp. 1081. Bacilli found in different sections from infected mouse footpads show lipoidal inclusions occurring as single (A) or lobed (C) units. The cell walls and the cytoplasmic membranes are intact but most of the cellular organization is gone (A–C). (×40,000; bar = 0.5 μm.) See discussion, Intracellular Storage of Lipids and Stages of Mycobacterial Growth.*

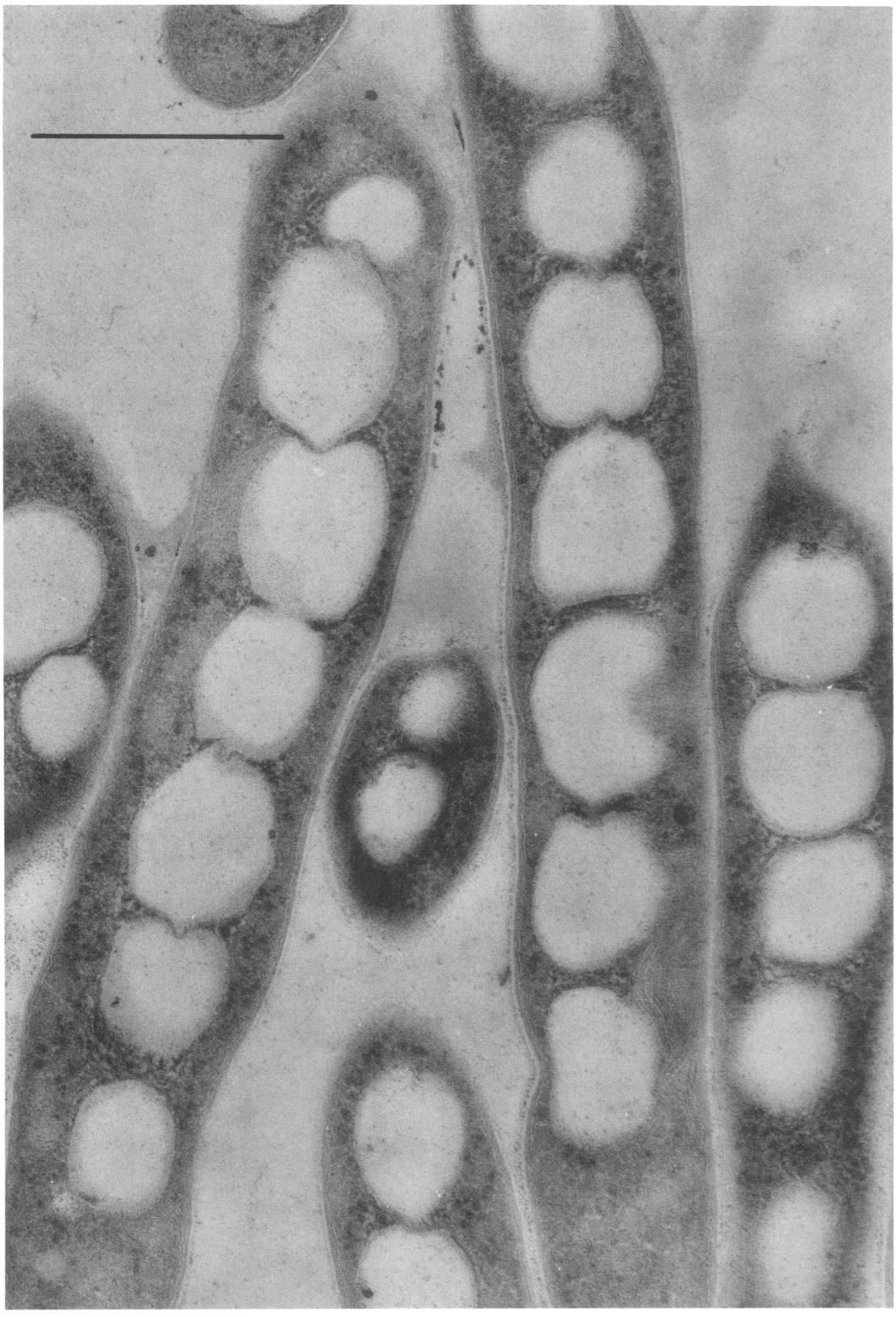
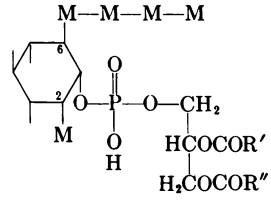


FIG. 13. *Section of old cells of Mycobacterium sp. strain ICRC, showing large fat inclusions, polysomes, and densely staining DNA (around the inclusions). See also Kölbel (629) for fat inclusions. (×78,000; bar = 0.5 μm.) See discussion of Intracellular Storage of Lipids.*

where R(I) = H, R(II) = mannosyl, R(III) = dimannosyl, R(IV) = trimannosyl, R(V) = tetramannosyl, and R(VI) = pentamannosyl (710). These phosphatidylinositol oligomannosides will be referred to as PI-Man$_x$. According to Lee and Ballou (709), the dimannosides are mannosylated at C2 and C6. All further mannosyl additions are at C6. Therefore, the pentamannoside, formula VI, has the structure:



It has subsequently been suggested by Brennan and Ballou (180) that, among the mannosyl derivatives of *M. phlei*, some are acylated on the mannose or myoinositol portion of the molecule. This is in addition to the acyl groups (R′ and R″) on the glycerol moiety of these phospholipids. The chemistry of mycobacterial phospholipids has been reviewed by Asselineau (59), Pangborn (905), Lederer (704), and Goren (453). Phosphatidic acid levels in mycobacteria can be assumed to be low, of rapid turnover, and confined to the cytosol. Although the major phospholipids are reviewed here in conventional catalog fashion, their actual state is certainly dynamic and ever-changing in the biosynthetic processes of the actively growing mycobacterial cell. This is suggested by their changing cellular distribution as populations move from logarithmic to interphasic states of growth. From the laboratory of Nojima have come findings relating the three mycobacterial phospholipids to the anatomy of the mycobacterial cell (20–22).

Cardiolipin

In mycobacterial CL, the distribution of FAs was nonrandom: the unsaturated acids, octadecenoic and tuberculostearic, were located at the α-position, whereas palmitic and other saturated FAs were located mainly at the β-position. There was no evidence for the presence in CL of such long-chain FAs as phthienoic, mycocerosic, or mycolic. The patterns of the FA (acyl) substituents in the CLs differed from species to species (*M. tuberculosis* Aoyama B, *M. bovis* BCG, *M. phlei* Penso, and *M. butyricum* [896]). The turnover rate of CL in *M. phlei*, log phase, was high (22). CL seemed most concentrated in the cytoplasmic membrane fraction (21). No phospholipid of any class was detected in the fluid cytoplasm (minus membranes and ribosomes; P content of this fraction was 1.1%).

Phosphatidylethanolamines and Glycosyl Diglycerides

The distribution of PE was more difficult to pinpoint (21). It was most concentrated in the cytoplasmic membrane fraction and in fractions that were mixtures of ribosomes and cytoplasmic membranes. Minnikin et al. (824) have suggested a possible interchangeability of PE and diglycosyldiglycerides in the membranes of some members of the genus *Bacillus*. Whether this means that the two serve similar functions remains to be discovered.

Schultz and Elbein (1035) have reported the transfer, by a particulate system from *M. smegmatis*, of [^{14}C]glucose from UDP-[^{14}C]glucose and [^{14}C]galactose from UDP-[^{14}C]galactose into mycobacterial acceptors (endogenous) to yield monoglycosyldiglycerides and diglycosyldiglycerides. The major FAs of these glycerides were palmitic and oleic. Some stearic acid was also present. The authors suggest that these diglycerides are membrane bound and therefore may serve as carbohydrate reservoirs or function in the transport of hexose residues across the membrane (see also [1049]).

Mannophosphoinositides

The PI-Man$_x$'s of *M. phlei* were most concentrated in the cell wall fraction. In growing *M. phlei*, they seemed slow to turnover. Yet, measured as mannose of PI-Man$_x$, the phosphatidylinositol mannoside family of compounds is also found, though at much lower concentrations, in the membrane fraction(s). This would be expected if shorter precursor molecules were associated during biosynthesis mostly with the cytoplasmic membrane fraction or if PI-Man$_x$'s always were in continuum with their points of origin. Homogeneous layers are probably the exception in bacterial organization. There are probably always intergradations and intrusions from one layer into another. It seems reasonable to consider phosphatidylinositol oligomannosides as essential cell wall components of mycobacteria (see also Motomiya et al. [858] regarding the location of PI-Man$_x$ in the walls of *Mycobacterium* sp. P-6). Kotani's early hunch that they might function as a matrix in the wall (653) seems reasonable, and proof of such a function would be welcome. Nojima (879) isolated from a "wax D" of *M. bovis* BCG, a phosphatidylinositol polymannoside which, on deacylation, gave a mixture of glycerophosphorylinositol oligomannosides with two, four, and five mannosyl residues. This is strong additional evidence that PI-Man$_x$ is a cell wall component. Goldman reports from studies with H_{37}Rv that phosphatidyl-*myo*-inositol dimannoside occurred in the *cytoplasm*, cytoplasmic

membrane, and cell wall. However, he reported that the cell wall contained the greatest concentration of di- and pentamannosides (436). The PI-Man_x's can be extracted with pyridine (905). Evidence for a pyridine-extractable matrix-like material extending from points just beneath and through the murein layer into the outer membrane of *M. smegmatis* 607 is provided in Fig. 8A. Cells, after extraction with pyridine, become sticky and tend to agglutinate. They retain their acid-fastness. Not only are they acid-fast, but also, carbol fuchsin penetrates them more readily than it penetrates their unextracted counterparts. As shown in Fig. 8B, extraction with alkaline ethanol removes the peptidolipid layer. Such cells no longer are acid-fast. Pyridine extraction leaves the peptidolipid layer essentially intact except for random areas, where it removes pyridine-soluble material that had extended through the peptidolipid. The pyridine-extracted substance(s) is shown in Fig. 9. Kataoka and Nojima have suggested that PI-Man_x may be very special phospholipids of the *Actinomycetales* (588). They have pointed out that the phospholipids of *C. diphtheriae* (58, 439), *N. asteroides* and *N. brasiliensis* (690), *M. bovis* BCG, *M. phlei* (905), and *M. smegmatis* (681, 1119), and *Streptomyces griseus* and *Microbisporangium chromogenes* (588) all contain PI-Man_x.

Biosynthesis of Mannophosphoinositides

Brennan and Ballou (179, 180) have addressed themselves to the biosynthesis of the dimannophosphoinositides in *M. phlei*. Phosphatidyl-*myo*-inositol can act as an acceptor in a test tube system where mannose is donated as guanosine 5'-diphosphate mannose (GDP-man) (512). The requisite enzymes occurred in (both the soluble and) the particulate fractions of *M. phlei*. The dimannophosphoinositides were multiply acylated (two fatty acids were attached to the glycerol moiety and additional FAs were esterified to available hydroxyls on the mannose or *myo*-inositol portions). The acylation step required CoA and adenosine 5'-triphosphate (ATP) but could be effected by fatty acyl-CoA. The specificity of the enzyme was not limited to fatty acids found in *M. phlei*. It could add on not only palmitic and 10-methylstearic (tuberculostearic) acids but also myristic, stearic, and oleic acids. This latter information may be of considerable interest when comparative lipid biosynthesis is assessed. The pathway discussed by these authors offered two possible routes from phosphatidyl-*myo*-inositol to dimannophosphoinositide: (i) monomannophosphatidylinositol accepts mannose from GDP-man at C6 of the inositol and is then acylated to become dimannophosphoinositide or (ii) monomannophosphatidylinositol becomes acylated in the inositol ring, after which it becomes mannosylated in position 6 on the *myo*-inositol ring, yielding a dimannophosphoinositide acylated in the inositol part. Takayama and Goldman (1121) found that a particulate fraction from $H_{37}Ra$ incorporated radioactive label from [^{14}C]mannose only at position 6 of the dimannoside and at position 2 in the monomannoside. Two reactions seemed to occur:

(i) phosphatidyl-*myo*-inositol + GDP-D-[1-^{14}C]mannose → *myo*-inositol mono-[1-^{14}C]mannoside + GDP

(ii) phosphatidyl-*myo*-inositol monomannoside + GDP-D-[1-^{14}C]mannose → phosphatidyl-*myo*-inositol di-[1-^{14}C]mannoside + GDP

To these authors, this finding of label at C2 in the monomannoside and *only* at C6 in the dimannoside precluded the possibility that the monomannoside might be the direct precursor of the dimannoside. However, they suggested that once monomannosides were acylated on the *myo*-inositol ring they might serve as acceptors at C6. These findings fit in with the general information about PI-man_x. The work of Lee and Ballou (90, 709, 710), Hill and Ballou (512), and Pangborn and McKinney (906) had indicated that *M. phlei* and *M. tuberculosis* produced a variety of dimannosides that differed as to the number of their fatty acyl groups. Dimannosides having 4, 3, and 2 mol of FA per mol of phosphate have been found in both *M. tuberculosis* and *M. phlei*. Probably route ii above (acylation in the *myo*-inositol ring of the monomannoside being a prerequisite for mannosylation at position 6) is central to the formation of the more acylated and mannosylated PI-Man_x's. Different species of mycobacteria may possess mannosyltransferases and fatty acylating enzymes of varying specificities and these, plus intracellular restrictions as to the kinds of FAs available for addition, could account for the wide variety of PI-Man_x's synthesized by different mycobacterial species (in vivo), as well as for limitations on in vitro systems. Takayama and Armstrong have found the cell-free system of *M. smegmatis* to yield PI-Man_1, PI-Man_2, PI-Man_3, PI-Man_4, and PI-Man_5, but PI-Man_4 was most abundant, and PI-Man_2 was least abundant. This contrasted with the cell-free system of *M. tuberculosis* and *M. phlei* which, as indicated above, yielded only PI-Man_2 (1119).

Oka et al. (894) have agreed with the findings

of others regarding the cellular distribution of phospholipids, but they have inferred that the constitutively (scoto-) chromogenic strain, P-6 from Runyon, has as its major phosphatidylinositol mannoside not PI-Man_x but phosphatidylinositol monomannoside, thus implying an inherent difference regarding the lipids of "unclassified" mycobacteria (Runyon group II). Khuller and Brennan (611) have been quick to demonstrate that monomannophosphoinositides were in fact absent in strain P-6, which they found to have a preponderance of dimannophosphoinositides. So far, then, dimannophosphoinositides seem to be of general distribution among the mycobacteria.

Kataoka and Nojima advance the idea that the proportion and kinds of the phospholipid triad, CL, PE, and PI-Man_x, in the *Actinomycetales* are unique. Although phosphatidylinositol mannoside and CL are found in corynebacteria, Brennan and Lehane report that PE is absent in *C. diphtheriae* (182), although Khuller and Brennan have reported it in an organism referred to as *Corynebacterium aquaticum* (610), and Komura and associates have found traces of PE in *C. diphtheriae* (632).

Immunizations with Cardiolipins, Phosphatidylethanolamine, and Phosphatidylinositol Mannosides

In studies of an unclassified constitutively chromogenic mycobacterium, P-6 (obtained from E. H. Runyon), Motomiya et al. (859) showed that the CL fraction from P-6 behaved as a satisfactory antigen in the flocculation test for syphilis and adsorbed onto latex particles employed in an agglutination test using sera from patients with lepromatous leprosy. CL, PE, and PI-Man_x fractions were separately used for immunizing guinea pigs, and these animals were subsequently challenged with living $H_{37}Rv$ (7.5×10^4 bacteria). No protection was afforded by either of the three immunizing antigens. Although the capacity of leukocytes derived from animals immunized with CL, PE, or PI-Man_x to phagocytize $H_{37}Rv$ was examined in slide culture, there was no significant difference between cells derived from the control and the immunized animals (857). Pigretti et al. have reported that two PI-Man_x fractions from *M. tuberculosis*, when used to immunize mice, failed to extend the survival time after challenge with *M. tuberculosis* (931). Khuller and Subrahmanyam have reported that phosphatidylinositol mannosides, when combined with incomplete Freund adjuvant (IFA), elicit antibody formation in rabbits (613). Employing the kaolin agglutination test of Takahashi, Subramanyam and Singhvi (1105) have shown that phosphatidic antigens (identified by them as "PI-Mans") reactive with human tuberculous sera occur in $H_{37}Rv$, $H_{37}Ra$, *M. avium*, *M. phlei*, and strain 607, and these seem very closely related or indistinguishable from the antigen of Takahashi.

Portelance and Asselineau have called attention to the fact that fractions of phospholipids from BCG and $H_{37}Rv$ characterized as phosphatidylethanolamine and mannosides of phosphatidylinositiol were contaminated with lipids containing amino acids (sometimes devoid of phosphorous), lipopolysaccharides, and peptides (934). The biological properties of these contaminants might be confusing. Purity of preparations with name designations such as peptidoglycans, glycopeptides, peptidolipids, etc. (e.g., see section on Antigens), pose serious problems throughout mycobacteriology.

Lipids of Transfer, Carrier Lipids

The murein of the mycobacterial wall has much in common with other bacterial peptidoglycans (see section, Cytoplasmic Membrane to Peptidolipid). It is therefore to be expected that, in its biosynthesis, mechanisms common to a number of other bacteria should operate. Important in the biosynthesis of bacterial cell walls are membrane carrier lipids such as undecaprenol phosphate, $H-(CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2)_{11}-PO$ (38, 511, 794), which, through its phosphate, accepts P-MurNAc-pentapeptide from UDP-MurNAc-pentapeptide. Similar carrier lipid function is involved in the biosynthesis of lipopolysaccharides of gram-negative bacteria (1238, 1284, 1285). Schultz and Elbein (1034) have examined the biosynthesis of mannosyl- and glycosyl-polyprenol phosphates by particulate enzymes from *M. phlei*. The authors have suggested that such lipids may have a role in the buildup of arabinomannans of the mycobacterial cell wall. Takayama and Goldman (1122) have found (using $H_{37}Ra$) that 60% of the mannophospholipids formed by a particulate transmannolase acting with labeled mannose and mycobacterial phospholipid was [^{14}C]mannosyl-1-phosphoryl-decaprenol. Two alkali-stable mannophospholipids from *M. smegmatis* have been investigated by Takayama and Armstrong (1119). More recently, Takayama et al. (1124) have characterized these transfer lipids as mannosyl-1-phosphoryl-octahydroheptaprenol and -decaprenol. The importance of isoprenoid phosphates in the translocation of other microbial membrane subunits such as teichoic

acids has been discussed by Baddiley and coworkers (39, 1224). They have reviewed specific cases in vitro where carrier lipids derived from gram-positive organisms function in the transfer of capsular polysaccharides in systems from gram-negative organisms. Similarly, carriers from *Aerobacter* have been shown to function in the synthesis of mannans by crude extracts from *Micrococcus lysodeikticus* (682). Even though the lipid intermediates are known to exhibit minor differences as, for example, in the linkages of hexose units, it appears that their polyisoprenol residues are identical (682). It is possible, therefore, that carrier lipids from mycobacteria might function in in vitro systems from either gram-positive or gram-negative organisms.

Mycobacterial Methylations

Mycobacterial products possessing substituent methyl groups include methyl branches on FAs (i) produced via methylmalonyl-CoA or (ii) donated from *S*-adenosylmethionine. Methylated sugars are not uncommon among mycobacterial lipopolysaccharides. The restriction and modification systems, known to exist in mycobacteria (see section, Mycobacteriophages) but as yet undefined, undoubtedly involve the methylation of DNA. Cantoni (216) has reiterated his proposal of two decades ago that *S*-adenosylmethionine is the sole donor of methyl groups in methyl transfer reactions, with the possible exception of N^5-methyltetrahydrofolate involvement in the methylation of catechols and indoleamines in animals. Mudd (861) has suggested that, since the de novo synthesis of methionine requires methyl-B_{12}, examples may yet be found where the methyl group from B_{12} (which originates from the β-carbon of serine or other C_1 sources) itself might be donated directly rather than via *S*-adenosylmethionine.

Detailed information has long been available concerning certain methylation reactions in mycobacteria. Lennarz et al. have found the pattern of tuberculostearic (10-methylstearic) acid synthesis in *M. phlei* to go from stearate to oleate to 10-methylstearate. The conversion of stearate to oleate is oxygen dependent. When labeled oleic acid was provided to growing cells of *M. phlei*, it served as a "precursor" of 10-methylstearic acid, and the methyl groups were shown to derive from labeled methionine (715). The formation of the methyl side chain in 10-methylstearic acid apparently resulted from addition of a methyl group to unsaturated C10 of oleic acid. Jauréguiberry, et al. (546) examined the 10-methylstearic acid synthesized by *M. smegmatis* grown in the presence of methionine-methyl-D_3, and found no mass spectrometric evidence for trideuterated FA methyl esters. Molecular peaks corresponding to dideuterated compounds were observed. This suggested that during the transfer of the D_3-methyl groups, a proton was lost. Subsequently, Jauréguiberry et al., employing extracts of *M. phlei*, found evidence for an intermediate in the synthesis of 10-methylstearic acid, 10-methylenestearic acid, and they postulated that the loss of the proton in going from $-CD_3 \rightarrow -CD_2$ involved the migration of a hydrogen between C10 and C9 of oleate (548; see also [547]), with the subsequent loss of a methyl hydrogen in the formation of the methylenic side group (as in Fig. 14). The migration of the methyl hydrogen was further studied by mass spectrometry of deuterated compounds provided to *M. phlei* (714). Lederer has reviewed, in some detail, these and other methylation reactions (701; see also Jauréguiberry et al. [547] and Law [697]).

In looking into the details of the reaction leading to the methylation of tuberculostearic acid at C10, Akamatsu and Law have sought the endogenous lipid acceptor, the actual methyl donor, and the hydrogen donor for the reduction of the 10-methylene group to a methyl group. They have used the crude enzyme system (supernatant from 100,000 × *g* extract of sonically disrupted cells) involved in the synthesis (including methylation) of 10-methylstearic acid by *M. phlei* (17, 18). The cleanest syntheses were obtained with acetone-treated preparations, which were relatively free of endogenous lipid substrates. In this cell-free system, oleic acid failed to stimulate the synthesis of 10-methylstearate derivatives.

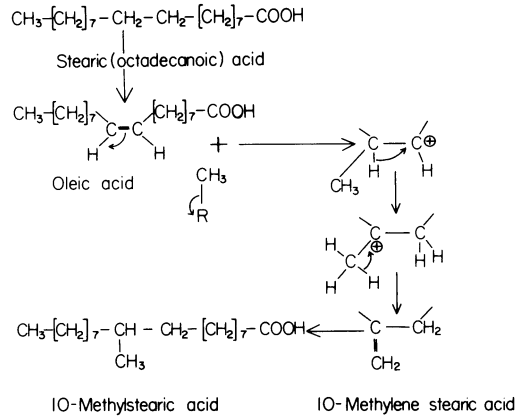


FIG. 14. *Synthesis of 10-methylstearic acid, after Akamatsu and Law (18). R = methyl donor.*

Olefinic fatty acyl phospholipids were required. The chain was alkylenated at the C10 to give a methylene group; this was discovered by employing *S*-[*methyl*-^{14}C]adenosylmethionine (methionine did not function as an effective methyl donor when added to the cell-free system), and this methylene group was subsequently reduced to a methyl group. Although the necessary methodologies for unraveling the exact role of the phospholipids were not available, the overall reaction appeared to encompass two steps:

(i) (olefinic fatty acyl) phospholipid + *S*-adenosylmethionine → (methylene acyl) phospholipid + *S*-adenosylhomocysteine

(ii) (methylene acyl) phospholipid + NADPH + H^+ → (methyl acyl) phospholipid + $NADP^+$

Phosphatidylglycerol, phosphatidylinositol, and PE could serve as substrates. Although the enzyme system was associated with the soluble fraction, there was enough activity in the membrane fraction to suggest that the *intact* system might be membrane associated. It has previously been pointed out that tuberculostearic acid and cyclopropane FAs accumulate in the stationary phase of growth (715). This has suggested to Akamatsu and Law that both of these processes involve enzymes that act upon the membrane phospholipids, alkylenating them after their deposition by phospholipid synthetases. Tuberculostearic acid may occur in a number of macromolecules of the mycobacterial cell. For example, Pigretti et al. reported that the phosphatidylinositol pentamannoside of *M. tuberculosis* contained 43% of this 10-methylstearic acid, along with large amounts of palmitic and small amounts of oleic and stearic acids (931).

Akamatsu and Law have also recovered a soluble enzyme from *M. phlei* that transfers methyl groups from *S*-adenosylmethionine to the carboxyl group of FAs. The role of this alkylation of carboxyl groups to yield FA methyl esters in the biosynthetic processes of *M. phlei* is not known (19).

Lornitzo (734) has reported protein bound 1-*O*-methyl-*sn*-glycerol-3-phosphate as a probable intermediate in a transmethylation reaction occurring in $H_{37}Ra$, leading to the formation of 1-*O*-methyl-glycerol from *S*-adenosylmethionine and *sn*-glycerol-3-phosphate.

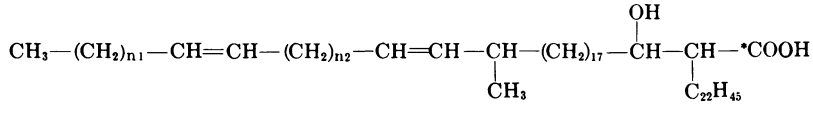
In Fig. 11 is shown a lipopolysaccharide, MGLP IV, which contains an α-(1 → 4)-D-gluco-oligosaccharide methylated in position 6 of each of the glucose units. A polysaccharide methyltransferase that brings about the transfer of these methyl groups from *S*-adenosylmethionine has been found in extracts of *M. phlei* (371, 470). Oligosaccharides of 7 to 10 glucose units in α-(1 → 4) linkage are suitable substrates; analogous structures in β-(1 → 4) linkage are not. In fact, the latter inhibited the reaction. The presence of acyl groups on the polysaccharide (Fig. 11) enhanced methyl transfer (607, 869, 1179).

Lipid Syntheses and Stages (Phases) of Mycobacterial Growth

Here and there in this review it is noted that metabolic products characteristic of aging populations are different from those of populations in log phase of growth. Donets et al. (310) have noted an increase in phospholipids of *M. album* from 9.3% towards the end of log phase to 15% in stationary phase; for *M. rubrum*, phospholipids increased from 15.5 to 19.2%. For both species triglycerides dropped from a peak (*M. album*, 20.4 to 15.4%; *M. rubrum*, 36.6 to 26.6%) at 6 days to a lower value at 12 days. The highest levels of free FAs were found associated with end-phase populations. Amounts of digitonin-precipitable lipids were higher in log-phase cells, reduced to 50% in late log, and increased at the onset of stationary phase. Although the authors refer to the latter as sterols, no data beyond their digitonin precipitability are offered to support this conclusion.

Mycolic Acids

The characterization of the corynomycolic acids (706, 949) and studies on their in vivo synthesis using [1-^{14}C]palmitate has indicated that parts of two molecules of palmitic acid enter into the biosynthesis of one molecule of corynomycolate, since label was found in C1 and C3 of the latter (421, 422). Etémadi and Lederer (356) have provided labeled tetracosanoic acid, $C_{23}H_{47}$-*COOH, to cultures of *M. smegmatis*, growing in Sauton medium, and then isolated from those cultures:



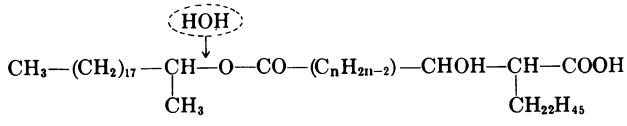
n_1 = 15–19; n_2 = 12–16

(α-smegmamycolic acid labeled in the carbon of the carboxyl group). To other growing *M. smegmatis* they supplied [*methyl*-^{14}C]methionine and found that the carbon of the methyl side chain was derived from the labeled methionine. These experiments, with their elegant supportive degradation studies, indicate that the final stage in the synthesis of α-smegmamycolic acid involves a condensation of the carboxyl terminal of a methyl branched FA (synthesized by *M. smegmatis*) with the α-carbon of the labeled tetracosanoic acid (supplied in the medium). As a result of this event, the carboxyl carbon during the process of condensing becomes the hydroxylated β-carbon of α-smegmamycolic acid. As mentioned above, the origin of the radioactive methyl group in the long-chain FA moiety (synthesized by *M. smegmatis*) was labeled methionine. Lederer has reviewed some of these methylation reactions (700, 701, 703).

Using [^{14}C]palmitic acid in combination with a cell-free extract prepared (in 0.05 M phosphate buffer, pH 7.0, plus 3×10^{-3} M mercaptoethanol and 0.01 M disodium EDTA) from a nontoxinogenic derivative of *C. diphtheriae gravis*, Halifax strain, Walker et al. (1217) recently have reported a Claisen sort of condensation of 2 mol of the labeled acid to yield 2-tetradecyl-3-keto-octadecanoic acid. With $NaBH_4$, these investigators were able to quantitatively reduce the β-keto acids, yielding a mixture of corynomycolic acids. Whether or not a buffer of greater ionic strength might have expanded the biosynthetic capabilities of these extracts is not known. (In this connection, see discussion of FAS I of *C. diphtheriae*.) More recently, Promé et al. (944) have found that under slightly modified conditions, from 2 mol of palmitic acid, the *C. diphtheriae* system synthesized a monotetradecyl-2-keto-3-octadecanoate of trehalose. This finding suggested to the authors that trehalose-lipid is an intermediate in the synthesis of corynemycolate.

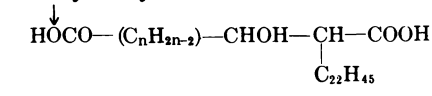
It would seem that the in vivo experiments of Etémadi and Lederer could be extended in the following way to give more information about the biosynthesis of mycolic acids in the CMN group. The close interrelations of the group are generally acknowledged (59, 159, 271, 441, 446, 653, 698, 699, 704) and have previously been discussed by one of us from the standpoint of the type species of the genus *Corynebacterium*, *C. diphtheriae* (96). A gradient of organisms from *C. diphtheriae* through *N. rubra* and *N. asteroides* to *M. bovis*, *M. kansasii*, *M. intracellulare*, *M. smegmatis*, and *M. phlei* could be arranged on the basis of the relative complexities of their mycolic acids (in this connection, see the findings of Maurice et al. concerning variations in mycolic acids of nocardias [798] and discussions of Etémadi on phylogenetic implications of various mycolic acids [352–354]). Since each organism in the list produces α-alkyl, β-hydroxy acids (mycolic acids), each is capable of joining the two branches. If *M. smegmatis* or *C. diphtheriae* were supplied labeled $C_{25}H_{51}$-*COOH synthesized by *M. tuberculosis*, would they produce mycolic acids with a $C_{24}H_{49}$–α-branch? Presumably, they would. Both *C. diphtheriae* and *M. phlei* possess FASs I and II. The chain lengths of the β-hydroxylated moiety of their mycolic acids are very different (59). Further comparative study of these two systems would enhance our understanding of mycolic acid biosynthesis.

Etémadi and Gasche have reviewed FA products of mycobacteria that might represent intermediates in the biosynthesis of mycolic acids and have considered the origin of the components of the dicarboxylic mycolic acids found in *M. avium* and certain other species (355). They suggested that precursors of the dicarboxylic acids might be keto mycolic acids and that the final intermediate might be an ester resulting from the introduction of an oxygen between the keto carbon and its neighbor (see arrow in the formula of the wax of Lanéelle and Lanéelle, below). Hydrolysis would then yield the dicarboxylic mycolic acid and a carbinol. This would mean that the number of carbons in the dicarboxylic acid would be less (than that of the precursor) by the number of carbons in the carbinol component removed by hydrolysis. Lanéelle and Lanéelle (691) have addressed themselves to the problem of finding the keto intermediate of the dicarboxylic mycolic acids among the great mixture of mycolic acids produced by Johne's bacillus (*M. paratuberculosis*). They have found (i) keto acids with 80 to 85 carbons, (ii) dicarboxylic mycolic acids with 58 to 65 carbons, and (iii) the proposed dimycolic acid intermediate of 78 to 85 carbons:



Lanéelles' wax n = 32, 34, 36, 37, 38, or 39

which they called a wax. The latter existed as a monoglyceride. The proposed formula for the dicarboxylic mycolic acid is:



n = 32, 34, 36, 37, 38, or 39

Matching arrows in the chain of the dimycolic acid with that of the ester intermediate indicate the proposed origin of the acid. There is left the residual methyl carbinol. A precedent for the kind of oxidation, which renders the keto mycolic acid into the wax form, is found in the utilization of 2-tridecanone by *Pseudomonas aeruginosa*. Molecules of $CH_3—(CH_2)_9—CH_2—\overset{\overset{O}{\|}}{C}—CH_3$ undergo two distinct changes: (i) some are reduced to form 2-tridecanol [$CH_3—(CH_2)_9—CH_2—\underset{\underset{OH}{|}}{CH}—CH_3$] and (ii) some are oxidized to form an ester, undecyl acetate [$CH_3—(CH_2)_9—CH_2—O—\overset{\overset{O}{\|}}{C}—CH_3$]. The latter is split into two moieties: 1-undecanol [$CH_3—(CH_2)_9—CH_2OH$] and acetate (388). For a discussion of the possible role of 5-enoic acids (C_{22}, C_{24}, and C_{26}) in the synthesis of mycolic acids, see discussion by Asselineau and associates (57).

Isonicotinic Acid Hydrazide and Synthesis of Mycolates

Isonicotinic acid hydrazide (INH) exerts some unspecified effect on mycolic acid synthesis in mycobacteria (1125, 1220, 1270) as indicated by decreases in α-mycolates, methoxymycolate, and β-mycolate within 60 min after exposure. Viability of the cells also slowly decreases. About 5.2 pmol of INH per 10^9 cells serves to inhibit mycolic acid synthesis by about 50%. Scanning electron microscopy (SEM) of $H_{37}Ra$ cells exposed to an amount of INH (0.5 μg per 5×10^8 bacteria/ml) that completely inhibits mycolic acid synthesis in 60 min revealed a dramatic change in the cells over a 24-h period, with a complete loss of outer membrane areas L_1 and L_2 (Fig. 4) and the development of thin spots in the cell wall, with concomitant bulging. Takayama et al., the authors responsible for the foregoing data, have cautiously stated: "the results of this study did not allow us to determine whether or not the morphological changes observed are caused by the INH inhibition of mycolic acid synthesis" (1126). Of course, SEM will pick up only the more gross changes occurring in INH-treated bacteria. The elimination, for example, of the L_1 peptidoglycolipid is an alteration in fine structure that can be picked up only under very special conditions (Fig. 5).

In reviewing the efforts at elucidating the mode of action of INH, Youatt (1298) has suggested that INH acts specifically to combine with an enzyme peculiar to INH-susceptible bacteria. If the enzyme is not one that is crucial to either transcription or translation, removal of INH should allow for recovery of the cell from the INH effect. Takayama et al. (1120) have been able to show that, up to a critical time (prior to 10 h postexposure), washing cells exposed to INH (0.5 μg/ml) allows the cells to recover the capacity to synthesize mycolic acids. The restoration of the capacity was a slow process, involving an 8-h lag period and requiring about 24 h of subsequent incubation. Without washing, INH-treated cells are able to undergo one division (1118). Further, Takayama et al. have obtained evidence that during the first 12 h of exposure to INH, the contents within the cytoplasmic membrane of the cell increase at a normal rate without an equal increase in cell volume (1123). The pigments produced by *M. tuberculosis* in the presence of INH must still be fitted into the story of the mechanism of INH-induced bacterial death (1299). For that matter, the hypothetical target enzyme, "mycolic acid synthetase" (1120), remains to be characterized. It has long been known that, following INH treatment, INH-sensitive mycobacteria become non-acid-fast, whereas INH-resistant mutants retain their acid-fastness (627).

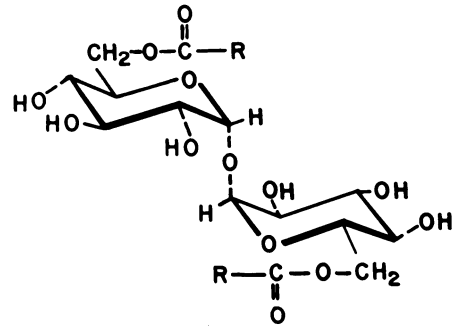
Acylglucose and Acyltrehalose

Winder et al. (1271) have found that *M. smegmatis*, under balanced conditions of growth with glucose as its carbon source, turned over free trehalose at a rate about 3 times that of its net formation, whereas the sugars of acylglucose and acyltrehalose are turned over at 13 and 8 times, respectively, their net formation. In the introductory section, we pointed out that acylglucoses are produced in large amounts only when glucose is provided as the sole carbon source. They have been detected in *Corynebacterium xerosis* (181), *C. diphtheriae*, and *M. smegmatis* (183). The major FA of acylglucoses from *C. diphtheriae* and *M. smegmatis* is corynomycolic acid. Brennan and his associates (1271) have suggested, from results obtained with radioactive glucose that, because of the turnover of acylated forms of glucose and trehalose (about 10 times per bacte-

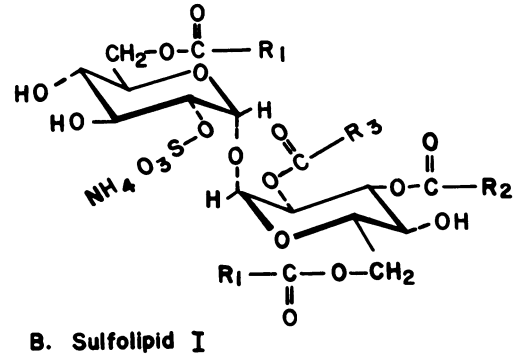
rial generation) under conditions of active growth, these sugars probably are not storage compounds. On the other hand, they do not seem to be structural compounds or, if they are structural compounds, they have some metabolic turnover as well. It seems to us that since mycobacteria under natural conditions rest sometimes and grow sometimes, always in the presence of their metabolic and catabolic products, it is possible that the mycolic acids and the sugars as acylated sugars may be in a sequestered state for use when needed. It is difficult to think of any natural environment in which 1% glucose would exist. Thus, we would favor regarding acylated sugars as storage compounds. Even the dimycolates of trehalose, which occur at the surface of cells and which behave as detergents, would probably serve as a source of energy when needed.

Cord Factor(s)

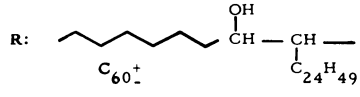
The toxic glycolipids containing trehalose, originally discovered by Bloch in petroleum ether extracts of *M. tuberculosis* $H_{37}Rv$ (141) and structurally defined by Noll et al. as dimycolates of trehalose (881), occur with distinctive modifications in members of the genera *Corynebacterium* (537), *Mycobacterium* (881), and *Nocardia* (536). The occurrence of these diesters of trehalose (see Fig. 15) is another common bond between members of the CMN group. It is interesting that, in *N. asteroides* and various true corynebacteria, the trehaloses are esterified with corynomycolic acids ($C_{28}H_{54}O_3$ to $C_{36}H_{68}O_3$), whereas in *Mycobacterium* (*Nocardia*) *rhodochrous*, the cord factors contain nocardic acids ($C_{38}H_{76}O_3$ to $C_{46}H_{90}O_3$). In *N. asteroides*, mycolic acids of the higher carbon numbers are found in the bound lipids of the cell wall but not in cord factors (536). Thus, from *Corynebacterium* to *Mycobacterium* via *Nocardia*, there is a gradient in total carbon number of these α-ramified, β-hydroxylated long-chain FAs from C_{28} to C_{90} and a gradual change in the macromolecules of the cell to which they are characteristically conjugated. (For exceptions see section on Acylglucoses.) A mycolate precursor, the β-keto C_{32} product of the condensation of two molecules of palmitate in the corynebacterial system (944), becomes converted into a monotetradecyl-2-keto-3-octadecanoate of trehalose. Winder et al. have found acyltrehalose undergoing rapid turnover in actively growing cells of *M. smegmatis*, suggesting to them, among other possibilities, that acylation might take place in the cell wall or membrane (1271). Thus, trehalose esters such as cord factor may be essential for the transfer of mycolic acids into the mycobacterial ultrastructure. Cord factors behave as detergents and this, coupled with the fact that they are located about the surface of the outer cell wall, suggests for them a role in facilitating the inward movement of certain molecules important to the growth of



A. Cord Factor



B. Sulfolipid I



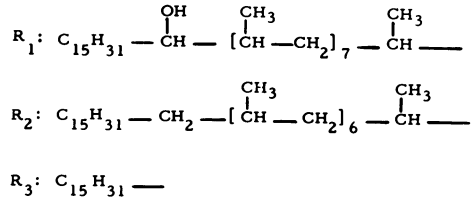


FIG. 15. *Two acyltrehaloses from Mycobacterium tuberculosis (after Goren [453]). For data concerning the spontaneous desulfation of sulfolipids, see (452). See also Tables 1, 2 and 3.*

the cell. Comparative uptake studies using mutants blocked and/or leaky in the synthesis of trehalose dimycolates could shed some light on the role of cord factor for CMN cells.

M. tuberculosis $H_{37}Rv$ elaborates the cord factor 6,6′-dimycolyl-α,α'-D-trehalose which, in microgram amounts, kills mice (601). The late Masahiko Kato has shown that this killing is associated with destruction of mitochondrial membranes and concomitant damage of the membrane-associated respiration and phosphorylation (592). Toida has found a side effect of cord factor intoxication upon the microsomal enzymes pyrazinamide deamidase and aminopyrine demethylase (1155). In contrast to mice, cord factor is relatively nontoxic for the rat (1013). (It has recently been discovered that there also accumulates around $H_{37}Rv$ a monomycolate of trehalose that is less toxic for mice than the dimycolate [600].) Asselineau and Kato have been interested in the essential requirements for cord factor activity. Early on, it appeared that ester linkages at the 6,6′ position of trehalose with mycolic acids and the presence of hydroxyl groups of the sugar in the molecule were essential to toxicity of cord factor (60, 63, 933). Mycolic acid alone or with trehalose, trehalose alone (591), acetylated cord factor, and 6,x-di-(3′-acetoxy,x-methoxymycolanoyl)-*N*-acetyl-D-glucosamine were shown to be nontoxic for mice and nondestructive for mouse liver mitochondria. On the other hand, 6-(3′-acetoxy,x-methoxymycolanoyl)-*N*-acetyl-D-glucosamine and methyl 6-(3′,x-sulfitomycolanoyl)-α-D-glucoside were toxic for mice and caused in vivo a decrease in the contents of mitochondrial protein, total lipids, and lipid phosphorous and a decline in the phosphorylative activity coupled to the oxidation of succinate in mouse liver mitochondria (592). These latter compounds appear to exert a detergent-like action on mitochondrial membranes. It was shown that two glycolipids prepared in the laboratory, methyl-6-mycoloyl-α-D-glucopyranoside and 6,6′-dimycoloyl sucrose, quantitatively retained the toxicity of cord factor (mean lethal doses [LD_{50}'s] of 187 and 161 μg, compared with 50 μg for the cord factor 6,6′-dimycoloyl-α-D-trehalose), and this indicated the possibility of constructing four stereoisomers of methyl-6-mycoloyl-α-D-glucopyranoside and examining their relative toxicity (598).

The subsequent study of Asselineau and Kato (61) has shown that the steric disposition of the hydroxyl groups at C2, C3, and C4 is essential for the toxicity of methyl-6-mycoloyl-glucoside, whereas the glycosidic linkage at C1 has no steric specificity. Peracetylated methyl 6-mycoloyl-α-D-glucopyranoside, like peracetylated cord factor, was without mouse toxicity and without effect on mitochondrial membranes as tested in vitro. (The acetylation of the mycolic acids of cord factor [58, 880] or those of cord factor analogues [59] had previously been shown not to affect toxicity.)

Since the tying up of the free hydroxyl groups on the trehalose fragment, either by methylation or acetylation, completely blocks toxicity, various investigators have suggested that the effect of the trehalose hydroxyls may be upon the fit of the glycolipid to the sensitive site on the mitochondrial membrane. This explanation finds an analogy in the toxic fungal octapeptide α-amanitin, which causes death through the inactivation of eukaryotic DNA-directed RNA polymerase II (379). Among the analogues of α-amanitin that are not toxic is amanullin (1255), a cyclic octapeptide having a second isoleucine in place of the γ-hydroxylated side chain found in α-amanitin. Here, again, the final fitness for biological activity involves specific hydroxyl group(s). Cord factor has been found to suppress pyrazinamide deamidase in livers of intoxicated mice but has no effect on the enzyme system in vitro, thus suggesting an effect on enzyme biosynthesis, probably secondary to destruction of mitochondrial membranes (1154).

Cord factor is not immunogenic, but it serves as a functional "hapten" when complexed with methylated BSA (MBSA). Cord factor-MBSA, in IFA, used as a vaccine, protected mice against both the toxic action of cord factor and infection with *M. tuberculosis* $H_{37}Rv$ (593). Protection was not afforded cord factor-MBSA-vaccinated mice challenged with either *Listeria monocytogenes*, *Brucella abortus*, *Salmonella enteritidis*, or *S. typhimurium*. This type of experiment had also been reported in 1973 (595). Antibody prepared in rabbits against cord factor-MBSA passively protected mice against the toxic action of cord factor. Cord factor–anti-cord factor antibody mixtures were nontoxic for mice. Anti-cord factor serum reacted almost as well with the trehalose-6,6′-dicorynomycolate of *C. diphtheriae* as it did with its homologous trehalose-6,6′-dimycolate from *M. tuberculosis*. It was nonreactive with sucrose-6,6′-dimycolate, methyl-6-mycoloyl-α-D-glucopyranoside, or 6,x-di-(3′-acetoxy,x-methoxymycoloyl)-*N*-acetyl-D-glucosamine. In "haptenic" inhibition studies, α-D-trehalose inhibited to almost 50% precipitation between cord factor-MBSA and its antibody. Precipitates were determined by both radioactivity and total protein, and the inhibition curves were almost superimposable one upon the other. Rabbits immunized with cord factor-MBSA remain sensitive to the injection of cord

factor, and this complicates skin test reactions related to cord factor in rabbits. Rabbits immunized with cord factor-MBSA showed no DH to tuberculin. (There was some reaction to MBSA.) BCG-vaccinated rabbits were tuberculin positive (594). It has subsequently been demonstrated that cord factor bound to neutralizing antibody loses all of its effects on mitochondrial processes but, once dissociated from immune complexes, it is again toxic (597). Kato examined the sera of man and animals infected with tubercle bacilli and found no antibody capable of precipitating cord factor from an emulsion. Similarly, mice and rabbits vaccinated with BCG or heat-killed $H_{37}Rv$ failed to develop precipitating antibody. Both mice and rabbits vaccinated with cord factor-MBSA responded with the formation of precipitating antibody. The rabbit antibody, which could be destroyed by mercaptoethanol, was recovered from immune precipitates and shown, by immunodiffusion against (i) goat anti-rabbit serum, (ii) goat anti-rabbit immunoglobulin M (IgM), and (iii) goat anti-rabbit IgG, to be IgM antibody (596).

The real cord factor. Goren has rued the fact that trehalose-6,6′-dimycolate has never been shown to be responsible for cording (453). Although the correlation between the capacity of *M. tuberculosis* to grow as serpentine cords and its ability to kill guinea pigs is very well established (283, 819), there is some question as to whether or not trehalose-6,6′-dimycolate is responsible for cording (454). Perhaps genetic studies could resolve this problem. A medium has been described which employs Triton WR1339 to give maximum expression of cording (733). Nitrosoguanidine has been shown to be a suitable mutagen for mycobacteria (639; see also section, Genetics). Since a loss of cording shows up as a change in colonial morphology, selection of hyper- and hypocording mutants should be simple. If it turns out that there is no direct relationship between cording and total yields of trehalose-6,6′-dimycolate, then the search for the real cord factor will certainly be undertaken.

Cord factor granulomas. The granulomas induced by cord factors are discussed in the section on Mycobacterium as Antigen.

Cord factor inhibition of tumors. Yarkoni et al. (1292) have reported that either trehalose-6,6-dimycolate, trehalose-6,6-dipalmitate, sucrose-6,6-dimycolate, or trehalose monopalmitate, when administered to mice subsequently challenged with Ehrlich ascites tumor cells, brought about a significant inhibition of tumor development (see also [117] regarding the suppression of urethan-induced lung adenomas in mice treated with cord factor). This induction of antitumor activity on the part of cord factor is suggestive of similar effects attributed to whole BCG (89, 148, 522, 696, 781, 991, 1029, 1055, 1308, 1309). Since the host response common to BCG infections and cord factor injections is the formation of granulomas (infectious agent granulomas in the case of BCG and foreign body granulomas in the case of cord factor), the antitumor activity, as has already been suggested (850), may well be in some way associated with the mobilized macrophage.

In the section, Ultimate Mycobacterial Adjuvant, it is pointed out that the unit, *N*-acetylmuramyl-L-alanyl-D-isoglutamine, in water-in-oil emulsion with ovalbumin, can enhance the production of specific antibody and the development of specific DH (655). Cell wall skeletons produce these effects and, in addition, when injected with neoplastic cells, promote the development of T effector cells cytotoxic, *in the test tube*, for the neoplastic cells (69). Recently, the adjuvant activity of highly purified cord factor (455) has been reinvestigated by an international group consisting of Saito, Tanaka, Sugiyama, Azuma, Yamamura, Kato, and Goren (1012), and it is clear that trehalose dimycolate in a water-in-oil emulsion does enhance both antibody production and the development of DH to egg albumin, hamster erythrocytes, and sheep erythrocytes (SRBC). Why this group did not also investigate the enhancement by purified cord factor of the development of cells cytotoxic for a specific neoplastic cell is not clear.

Sulfolipids

Goren has shown that the neutral red-binding strain $H_{37}Ra$ (817) produces families of structurally related sulfolipids (SLs) of which SL-I is a complex glycolipid ester having a molecular weight of about 2,386 and an empirical formula of $C_{145}H_{283}O_{20}NS$ (Fig. 15). It is a 2, 3, 6, 6′ tetraester of trehalose, which can be written as 2, 3, 6, 6′-tetraacyl-α,α'-trehalose-2′-sulfate (450, 451, 456). Goren et al. have suggested that neutral red binding may involve both the SLs and the phospholipids (PL) (457). The role of SL in the virulence of mycobacteria has been a major concern of Goren and associates. Kato and Goren have found that SL-I is nontoxic for mice. In vitro SL-I caused disruption of the membranes of mitochondria from mouse liver and inhibited mitochondrial oxidative phosphorylation. Various animal sera were found to neutralize these effects of SL-I in vitro. Presumably, they are capable of the same neutralization in vivo. The administration of SL-I, in

combination with cord factor, in vivo produced an enhanced damage of mitochondria over that of cord factor alone. Thus, mycobacterial sulfatides appear capable of augmenting the toxicity of cord factor (599). An examination of certain of the Mitchison strains of *M. tuberculosis* (which show reduced virulence for the guinea pig) for their combined capacity to produce SL and PL has suggested to Goren et al. (457) that there exists a "statistically very significant" correlation between virulence for the guinea pig and the capacity to produce SL and PL. A fraction of the attenuated strains, however, were hyperproducers of SL. Their capacity to produce other products such as cord factor was not measured. Although the $H_{37}Rv$ strain was used as a control in these experiments, the avirulent strain, $H_{37}Ra$, was not used. Perhaps in the near future we will have data comparing the cord factor/sulfatide ratio of selected virulent and avirulent strains. The spontaneous desulfation of purified SLs has been reported (452).

THE CYTOPLASMIC MEMBRANE SYSTEM

Mesosomes, Cytochromes, Iron-Chelating Compounds

A recent review of the literature of mesosomes leaves no doubt that these apparent organelles are in fact manifestations of the cytoplasmic membrane system (469). (The mesosome is visual evidence of excess membrane. Throughout nature, more-than-enough is the mechanism by which systems work: from the millions of sperm produced in excess of ova to be fertilized, to the excess of genes in terminally redundant phage genomes, such is the case. The emergent aspect of these excesses, whether in the findings of carbon balance studies of bacterial growth or the production of ears of corn in a field of corn plants, is an apparent exactness of systems.) It has previously been pointed out that in actively growing cells of the CMN group there is very little accumulation of excess cytoplasmic membrane as mesosomal structures (96), but that such accumulation does occur in late-log-phase cells. Asano et al. report this to be the case for cells of *M. phlei* (54). These workers found 97% of total cytochromes *b*, *c*, and $a + a_3$ in a membrane "ghost" preparation from *M. phlei*. Although oxidative phosphorylation in ghosts was only slightly lower than that found in electron transport particles (ETP; see review of intermediary metabolism by Rhamakrishnan et al. [958] and that of Goldman [435] regarding enzyme systems of mycobacteria), ghosts did not couple phosphorylation to oxidation. Most adenosine triphosphatase (ATPase) activity was associated with ghosts, as was malate-vitamin K reductase. The authors advance arguments for dual orientation characteristic of ghost membranes: (i) some properties suggestive of the inner membranes of mitochondria and (ii) permeability characteristics suggestive of a cytoplasmic membrane. Iron transport in *Mycobacterium* and *Nocardia* involves special transport molecules, nocardamin (609) and mycobactins. The latter have been the subject of an extensive review by Snow and White (1072; see also [96]). Ratledge and co-workers have suggested that the lipophilic mycobactin molecule is located on the "boundary layers" of the mycobacterium and that "it transports iron across the thick lipoidal layers of the mycobacterial cell" (748, 960) as ferric ion, and that the ferrous form is then released from its carrier by the action of NADP-linked reductase (192).

Macham and Ratledge (747) have also described a class of partly characterized water-soluble iron-binding compounds that they have termed "exochelins." These are reported to hold iron in solution at physiological pH values, are freely diffusible in the desferric and ferric forms, and can rob iron from ferritin. They can, thus, reverse the inhibition of mycobacterial growth by serum (625). Macham et al. (748) propose that iron in the extracellular milieu is solubilized by exochelins which, upon contact with the mycobacterial cell wall, are taken onto mycobactin, which then carries the iron through the mycobacterial wall (L_1, L_2, and L_3 of Fig. 4) to the cytoplasmic membrane where the NAD-linked ferrimycobactin reductase makes the ferrous ion available to the cytoplasm.

Recently, McCullough and Merkal have described the isolation of an iron-chelating secondary hydroxamate, L-α-asparaginyl-L-α-*N*-hydroxyasparagine, from cultures of *M. avium* and have reported the isolation of structurally related compounds from "corynebacteria" (802b). The enzymes associated with bacterial membranes have recently been reviewed by Salton (1014). The component of membranes especially pertinent to this review consists of the mycobacterial carotenoid pigments, which are covered in the next section.

Carotenoid Pigments of Mycobacteria

The polyene pigments, the carotenoids, are found in all groups of living things. They are synthesized only by plants, including microorganisms. Animals derive their carotenoids from

food. Although all photosynthesizing organisms contain these pigments, the distribution of carotenoids among fungi and nonphotosynthetic bacteria is far from universal (442, 1090). The genus *Mycobacterium* illustrates this point very well. Some species produce colorful polyenes (see Fig. 16), whereas others do not. Whether or not all mycobacteria have the capacity to produce the colorless phytoene (see Fig. 17), or earlier precursors of carotenoids, is

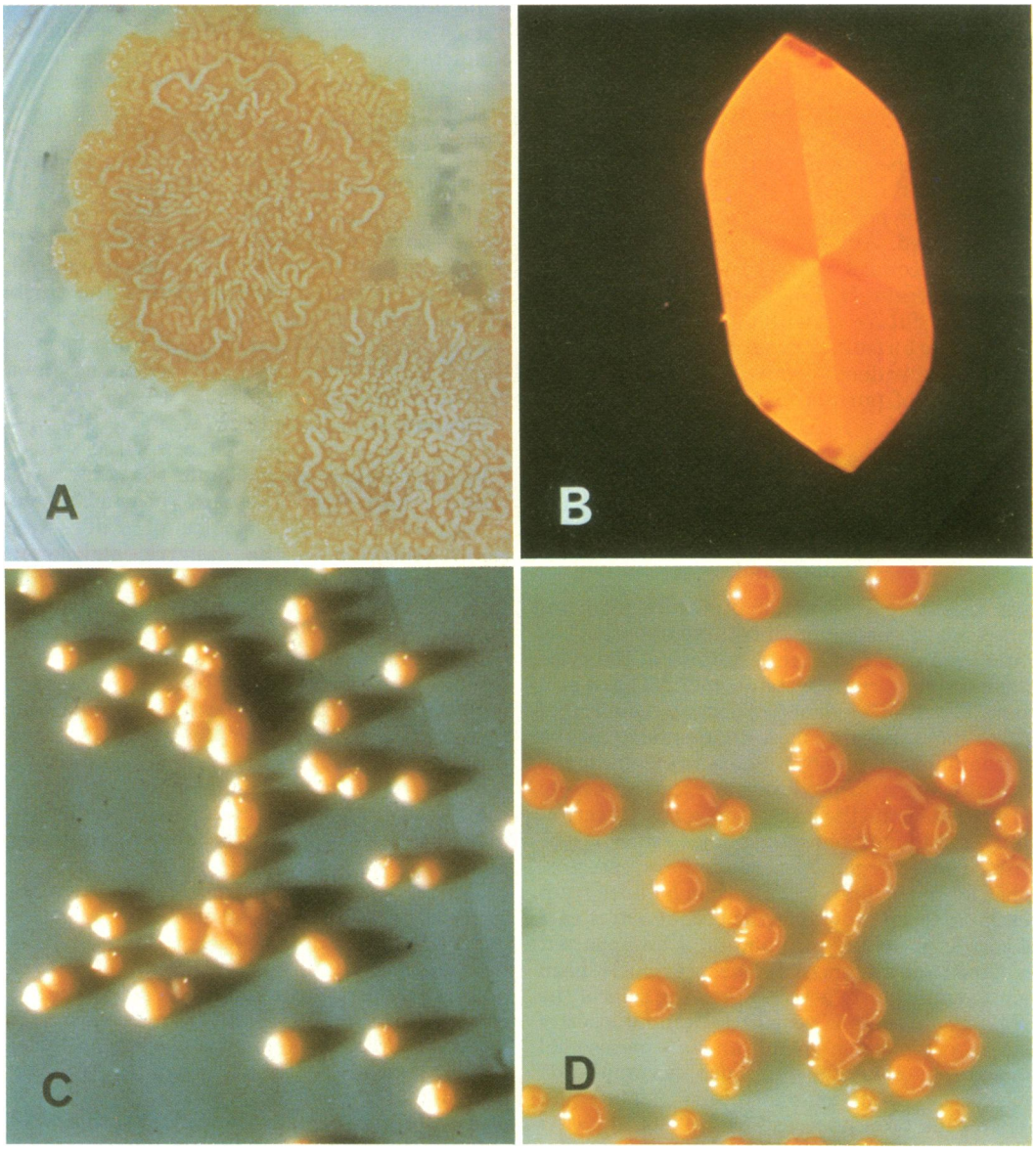


FIG. 16. *(A) Colony of Mycobacterium sp. growing on Dubos albumin agar. This mycobacterium has been called M. duvalii, see (1086). (B) Crystal of β-carotene. Kodachrome transparency by courtesy of Otto Isler (see 1236) and Hoffman-La Roche (Basel). Crystals of this carotenoid pigment accumulate around the colonies of certain strains of M. kansasii. For biosynthesis, see Fig. 17. For a discussion of the probable role of carotenoids in mycobacterial survival see section on Carotenoid Pigments. (C) Growth of a constitutively chromogenic mycobacterium on Lowenstein-Jensen medium. (D) Enhancement of color of strain in C following 2 weeks of exposure to light. Reproduced with permission from (1203) using transparencies kindly supplied by George P. Kubica. See Tables 1, 2, and 3.*

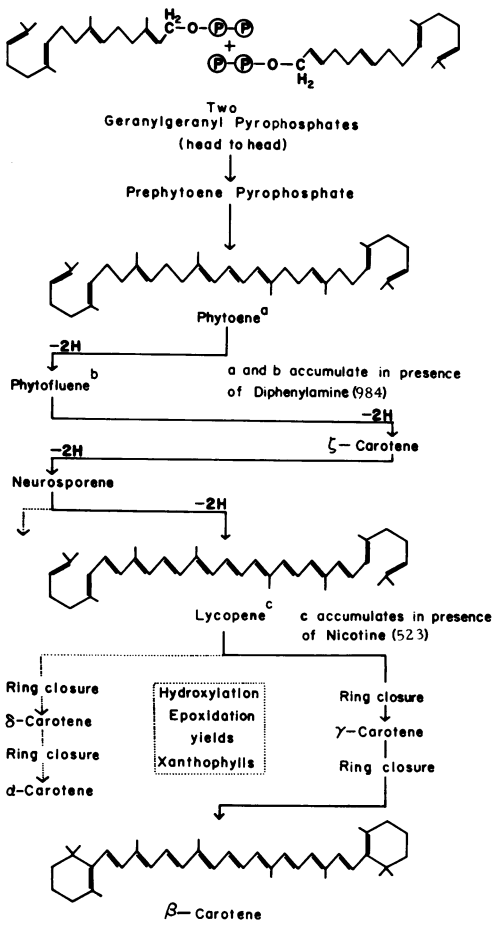


FIG. 17. *Probable biosynthetic pathway for β-carotene in Mycobacterium marinum. M. marinum also makes small amounts of α-carotene (106). The dotted lines indicate branch points which, in different species, lead to the synthesis of other carotenoids (e.g., α-zeacarotene) and xanthophylls. See Porter and Anderson (936), Batra (106) and Goodwin (444). Since this figure was prepared, David (287) has presented data concerning the "biogenesis" of carotenoid pigments in M. kansasii. He has chromatographic evidence to support the sythesis by M. kansasii of ζ-carotene, neurosporene, lycopene, leprotene, γ-carotene, δ-carotene, α-carotene, and β-carotene (288). Little is known concerning stereoisomerism and functional carotenoids of mycobacteria. According to Weedon, phytoene from carrots, tomatoes and the green alga, Chlorella vulgaris, have a 15-cis structure. The phytoene that accumulates in diphenylamine-inhibited Flavobacterium dehydrogenans appears to be a trans isomer; such is also the case with Mycobacterium sp. (469b). For a discussion of problems in stereoisomerism of carotenoids see Beytia and Rilling (130a) and Weedon (1236). See also Fig. 16 and section concerning Carotenoid Pigments of Mycobacterium. See Tables 1, 2, and 3.*

not known. Although colored polyenes were identified in mycobacteria some 40 years ago (238, 239, 534), their exploitation for mycobacterial taxonomy (568, 647, 994, 995) and the use of mycobacteria for elucidating the route of their own biosyntheses (106, 782, 982) is more recent.

Biology of Pigmentation

Among mycobacteria, pigmentation is a stable genetic property (568, 631, 878, 893, 994, 995, 1141, 1168, 1177, 1178). The carotenes of representatives of a single species appear to be alike. Tárnok and Tárnok (1141) have presented preliminary data showing this to be the case for the carotenoids of *M. phlei* (three strains), *M. avium* (two strains), *M. intracellulare* (eight strains), *M. aurum* (four strains), and perhaps *M. thamnopheos* (one strain). They found that the products of *M. marinum* (*balnei*, nine strains) and *M. kansasii* (seven strains) traveled as identical spots on thin-layer chromatography (TLC) plates. The same was true for those of *M. gordonae* (*aquae*, nine strains) and *M. scrofulaceum* (one strain). Pigmentation may be a *constitutive* attribute of a given strain (i.e., pigment is made under most, if not all, conditions suitable for growth) or pigmentation may be inducible (i.e., pigment production occurs only under conditions or following an event that leads to induction, e.g., exposure to light). Photoinduction of bacterial pigmentation was probably first reported by Prove in 1887 in an organism he called *Micrococcus ochrolecus* (945). In 1938, Baker demonstrated that incubation of *M. marinum* (*platypoecilus*) and 24 of 185 other strains of mycobacteria in subdued visible light resulted in an enhancement of their pigmentation (84). The presence or absence in a given species of *Mycobacterium* of the photoinducible trait has been successfully exploited in the taxonomy of the species (also called *photochromogens*) *M. kansasii*, *M. marinum* (*balnei* and *platypoecilus*), and *M. vaccae* (568, 994, 995, 1235), separating them from the *constitutively* pigmented strains (also called *scotochromogens*) and those species in which carotenoids are colorless or apparently absent.

Wild-type *M. kansasii* is photoinducible and, under certain conditions, accumulates, extracellularly, large amounts of crystalline β-carotene. A crystal of β-carotene is shown in Fig. 16B. Runyon has described constitutive (noninducible) mutants of *M. kansasii* that also accumulate large amounts of crystalline β-carotene (995, see also [997]). In studying a number of strains of *M. kansasii*, *M. marinum*, and *M. vaccae*, Runyon has come to the conclusion

that, in any clonally derived strain of *Mycobacterium*, color mutants (color *versus* colorless, inducible *versus* noninducible) are rare and stable. In other words, mycobacterial genes exhibit the universal stability of genetic matter. He has pointed out (994), as has Juhlin (568), that controlled conditions of illumination, oxygenation (no tightly capped tubes), and incubation are requisite to obtaining reproducible results regarding chromogenicity of strains. Thus, in order to exploit, for taxonomic purposes, carotenogenesis of mycobacteria, precise conditions relating to the biosynthesis of carotenes is a sine qua non. These have varied greatly from laboratory to laboratory (e.g., reference 878).

Tsukamura and Tárnok and Tárnok have given serious consideration to the effect of nutrition on carotenoid pigmentation in mycobacteria (1141, 1168). They have concluded that neither the source of nitrogen nor that of carbon affects pigmentation. Some media, such as those containing egg, give richer colors than solid Sauton medium. Of course, if the organism being tested gives poor growth on a given medium, its pigmentation will show up poorly. Tsukamura reports that old cultures of certain scotochromogens give increased coloration in response to the presence of sodium thioctate in the medium (1168).

Pigmentation and Virulence

When pigmentless mutants of pigmented strains were tested for their capacity to produce disease, it seemed that mutation to the colorless state was without effect on virulence of the organism or, for that matter, on other properties by which the organism(s) was identified (1177). Pigmentless mutants of *Staphylococcus aureus* exhibit no alterations in virulence. An examination of Tables 3 and 4 reveals several pigmented strains that produce disease in humans. Apparently, nonpigmented mutants of any of these strains would have the same disease-producing potential.

Biosynthesis of Mycobacterial Carotenoids

The lipid-soluble carotenes and xanthophylls typically contain eight isoprene residues of 40 carbon atoms. In 1950, Porter and Lincoln (937), studying carotenogenesis in mutant tomato plants, suggested that, in the biosynthesis of carotenoids, there was perhaps a progression from saturated to relatively desaturated molecules such as is shown in Fig. 17. Porter and Anderson (935) revised their scheme in 1962 at a time when strong support for it came from the investigations of Liaaen-Jensen, Cohen-Bazire, and Stanier in relation to the purple photosynthetic bacteria (724). Porter and Anderson reviewed additional data in 1967 (936) and summarized support for the probable pathways of the biosynthesis of carotenes from acetate to mevalonic acid to isopentenyl pyrophosphate to geranylgeranyl pyrophosphate. More recently, other aspects of carotenoids have been reviewed: their isolation (reactions) by Liaaen-Jensen (723), their total syntheses by Mayer and Isler (799), their synthesis in nonphotosynthetic plants by Batra (106), and their overall biosynthesis by Goodwin (443, 444).

The pathway shown in Fig. 17 for the biosynthesis of β-carotene by *M. marinum* probably applies equally well to *M. kansasii* and other carotenoid-synthesizing mycobacteria. Whether or not all mycobacteria have the machinery for making the precursors of carotenoids is not known. Presumably, synthesis begins with the ATP-linked decarboxylation of 5-pyrophosphomevalonic acid to yield isopentenyl pyrophosphate (IPP) and, through the action of isopentenyl pyrophosphate isomerase, 3,3-dimethylallyl pyrophosphate (DMAPP). Sequential head-to-tail condensation of these terpenes (DMAPP and IPP) yields, successively, geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate. The head-to-head condensation of two of the latter polyprenyl phosphates involves the formation of a C_{40} cyclopropylcarbinyl pyrophosphate ester, prephytoene pyrophosphate. The relatively saturated molecule, phytoene, is presumed to be formed directly from prephytoene pyrophosphate (29). By a series of desaturation steps, the increasingly unsaturated sequence, phytofluene, ζ-carotene, and neurosporene, leads to the formation of the tomato-red pigment, lycopene. In bacteria in which open-chain carotenogenesis has been induced with visible light, diphenylamine inhibits the desaturation steps beyond phytoene and phytofluene, resulting in the accumulation of these compounds. Rilling has suggested that diphenylamine and the diphenylketone, benzophenone, both of which block carotenogenesis in *Mycobacterium* sp. 1312, act by binding to dehydrogenating enzymes involved in the desaturation steps essential to the formation of open-chain carotenoids (984). Howes and Batra have discovered that the addition of 5 mM nicotine to photoinduced cultures of *M. marinum* results in an accumulation of lycopene and no β-carotene. When the concentration of nicotine was lowered to 0.5 mM, lycopene, some γ-carotene, and some β-carotene were formed. This strongly suggests that nicotine serves to block the cyclizations needed to yield the final major product, the

bicyclic polyene, β-carotene (523). Kleinig has reported that D- and L-nicotine are equally effective in bringing about the accumulation of lycopene in the case of *Myxococcus fulvus* (617). Once lycopene has accumulated, its conversion to β-carotene requires neither oxygen (107, 804) nor new enzyme protein synthesis (107). Recently Batra et al (107) have shown that 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) also blocks the cyclizations but, unlike nicotine, CPTA cannot be removed by washing of treated cells.

Runyon provided Tárnok with a mutant of *M. kansasii*, 3053, that accumulates lycopene. Tárnok and Tárnok (1142), working with 3053, have felt constrained to show that its lycopene is identical with lycopene from tomatoes (see Fig. 18). They have also shown that a red mutant of *M. kansasii*, Runyon's 3347A, forms mostly lycopene but produces traces of β-carotene. Both of these mutants would appear to be blocked in cyclization reaction(s). Number 3347A seems, in addition, to be a leaky mutant. A white mutant of *M. kansasii* (also from Runyon) examined by Tárnok and Tárnok accumulated phytofluene. Their preliminary studies indicate that mycobacterial mutants could be useful for delineating certain pathways of carotenoid biosynthesis. For example, the simple scheme shown in Fig. 17 offers the pathway of carotenogenesis in a *Mycobacterium* that makes one major pigment. A more complex scheme (see dotted lines) is required to accommodate to the capacities of *M. phlei* whose major products, the phlei-xanthophylls, require various oxygenating steps for their completion.

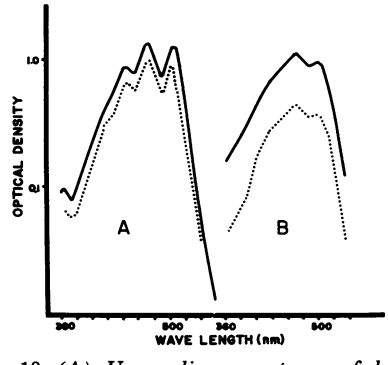


FIG. 18. *(A) Upper line, spectrum of lycopene from a mutant of M. kansasii. Lower (broken line), spectrum of lycopene from tomatoes. Solvent: petroleum ether. (B) Upper line, spectrum of β-carotene from M. marinum. Lower (broken line), spectrum of synthetic β-carotene. See also Fig. 16B and 17, and section on Carotenoid Pigments of Mycobacteria. Adapted from Tárnok and Tárnok (1142). See Tables 1, 2, and 3.*

One of these, phlei-xanthophyll (V), is a monocyclic, hydroxylated terpene (empirical formula, $C_{46}H_{64}O_7$) and is a tertiary glycoside of D-glucose (506). Conceivably, data on a variety of steps in carotenogenesis in mycobacteria involving such capacities as the ability to add on oxygens and hydroxyl groups, initiate cyclizations and isomerizations, incorporate glycosidic residues, etc. (all reflected in minor and major carotenes formed), could be of use in the chemotaxonomy of the genus.

Induction of carotenoid synthesis. The photoinducible strains differ from the noninducible strains in that, following exposure to visible light (400 to 700 nm), their level of carotenoid(s) biosynthesis is severalfold greater than their basal level. For example, Tárnok and Tárnok (1141) have shown that *M. kansasii* and *M. marinum*, growing in the dark, yield trace amounts of four different carotenoids. Photoinduced cultures, however, yielded large amounts (90% of the total carotenoids) of β-carotene. Constitutive (scotochromogenic) strains show slight stimulation by light.

Below saturating doses, the yield of carotenoids is directly related to the dose of illumination. A given dose of light leads to the same response independent of whether it is administered in fractions or in one continuous exposure. The amount of light required for induction varies from species to species. For example, Rilling (982) found *Mycobacterium* sp. to reach saturation by exposure for 1 to 1.5 min to 700 ft-c of light whereas, at the same light intensity, *M. marinum* was subsequently found to require 10 to 15 times as much illumination (983). Batra has reckoned the actual radiant energy required for a half-saturation response to be 5.3×10^4 ergs/cm^2 at 445 nm for *Mycobacterium* sp. and 3.6×10^5 ergs/cm^2 at 404 nm for *M. marinum* (106). The most effective wavelengths (action spectra) for the photoreaction vary from species to species. For *M. marinum*, Mathews reported important maxima at 410 and 439 nm and smaller ones at 502, 528, and 560 nm (782). For a discussion of these maxima in relation to susceptibility of cells to photokilling see the section on Protection Against Photoinduced Cell Death.

Zalokar showed that the synthesis of carotenoids in the red bread mold, *Neurospora crassa*, could be stimulated by light (1306), that oxygen was required for synthesis of carotenoids, and that diphenylamine inhibited carotenogenesis (1307). Acknowledging this information as a stimulus, Rilling, in 1962, reported a series of experiments with the photoinducible mycobacterium, *Mycobacterium* sp. 1312 (obtained from E. H. Runyon), in which he found a

pattern of photoinduction that closely paralleled that of *N. crassa*. In this and a subsequent paper (982, 983) he established that: (i) the photoinducible step required oxygen and light but was independent of temperature. Photoinduction did not occur in a nitrogen atmosphere (see also Howes et al. [525] and Tsukamura [1168]). (ii) There was a characteristic action spectrum for photoinduction, with maxima at 365 and 460 nm. (iii) The photoinductive step appeared to be a photooxidation requiring more than one quantum of light per bacterium, and the photoproduct was postulated to be capable of inducing a carotenogenic enzyme. Photoinduction took place at temperatures of 0 to 15°C and below. After storage of photoinduced cells at −15°C for 3 months, carotenogenesis occurred upon incubation in the dark at temperatures suitable for growth. Therefore, the initial photoproduct seemed very stable. (iv) During incubation after photoinduction, there was a sequential appearance of newly synthesized carotenoids; phytoene and phytofluene appeared first, followed by more highly unsaturated carotenoids. The addition of chloramphenicol at various points postinduction led to a differential inhibition of synthesis, which resembled the time sequence of appearance of the progression from saturated to less-saturated carotenoids (Fig. 17). These results led Rilling to postulate that the photogenerated inducer might (i) be a key enzyme that led to the sequential induction of other carotenogenic enzymes or (ii) derepress an operon encompassing the genes of carotenogenesis. Johnson et al. (553) have recently shown that prephytoene pyrophosphate synthetase is totally photoinduced in *Mycobacterium* sp. and that the constitutive levels of geranylgeranyl pyrophosphate synthetase found in dark-grown cells are increased after photoinduction. On the other hand, the levels of enzymes concerned with the synthesis of C_{35} to C_{40} polyprenyl pyrophosphates and isopentenyl pyrophosphate isomerase were unaffected by photoinduction.

Batra et al. have discovered (107) that a component (either A_1, A_2, A_3 or A_4 [109]) of the antimycin A complex induces carotenogenesis in *M. marinum* but not *Mycobacterium* sp. 1312. The induction is different from, and additive with, induction by light.

Protection Against Photoinduced Cell Death

The carotenoid pigments of mycobacteria have been shown to exert a protective effect against lethal photoproducts such as those associated with photodynamic killing by the dyes toluidine blue and eosin (782). This function of carotenoids in the neutralization of products of photodynamic action appears to be general among photosynthetic (331, 1058, 1090), and a number of nonphotosynthetic, bacteria (786). For example, white mutants of *Sarcina lutea*, blocked at some step in carotenogenesis, are rapidly killed by exposure to sunlight, whereas the fully pigmented wild type is not (788). Long ago, it was discovered that diphenylamine in small amounts would suppress the synthesis of carotenoids (1182, 1183), presumably at a step prior to the formation of phytoene (Fig. 17). Mathews exploited this diphenylamine suppression to show that a population of *M. marinum*, exposed to 10^{-4} M diphenylamine, 1.25×10^{-5} M toluidine blue, and 1,000 ft-c of light, was killed, whereas populations exposed only to (i) light and diphenylamine, (ii) light and toluidine blue, or (iii) diphenylamine and toluidine blue, under conditions of darkness, all survived and grew (782). Thus, carotenoids appear to protect *M. marinum* from the lethal effects of photodynamically activated *dyes*.

Using various carotenoid mutants of *S. lutea* as experimental organisms, Mathews-Roth and Krinsky (790) have shown that, in protection against photodynamic action (toluidine blue), there are at least two critical factors: (i) the actual amount of carotenoid synthesized *per cell* and (ii) the kind of carotenoids produced by the cell. Carotenoids having fewer than nine conjugated double bonds seem to be incapable of protecting against photodynamic killing. These data regarding conjugated double-bond content and protection for nonphotosynthetic organisms confirm earlier observations by Stanier (1089), Claes and Nakayama (249), and Crounse et al. (268) in photosynthetic bacteria.

Photokilling and photoinduction (of carotenoids) appear to be interlinked, possibly competing, processes. Data that strongly supported the idea that light-induced carotenogenesis and photokilling are competing processes catalyzed by a common endogenous sensitizer came from studies of photolysis of *Myxococcus xanthus*. Burchard and Dworkin (202) reported that *M. xanthus* grown in the light developed an orange carotenoid after the cells entered the stationary phase of growth; pigment content increased with age. (Mathews-Roth and Krinsky [790] also found more pigment per cell in stationary-phase cells than in cells in the log phase of growth.) Cells grown in the dark did not develop carotenoid(s) and could be photolysed by relatively low-intensity light (only during the stationary phase). The rate of photolysis increased with age. The action spectrum for photolysis of *M. xanthus* proved to be strikingly similar to the absorption spectrum for protoporphyrin IX (203). Purified protoporphyrin IX ex-

tracted from stationary-phase cells (which contained 16 times the porphyrin found in log-phase cells) was capable of sensitizing log-phase cells to photolysis (202). Burchard and Hendriks (204) have shown the maxima for the action spectrum for carotenogenesis in *M. xanthus* to be at 405 and 410 nm, with subsidiary maxima at 512, 533, 548, 585, and 635 nm. These are reasonably matched to the absorption spectrum of the tetrapyrrole, protoporphyrin IX; peaks in 25% HCl are at 409, 556, and 601 nm (959). Here, then, protoporphyrin IX is the endogenous photosensitizer. In logarithmically growing cells, the level of the tetrapyrrole is low. Exposure of such cells to light initiates the synthesis of photoprotective carotenes. Burchard and Hendriks have suggested that these highly unsaturated molecules may serve as a substrate for photooxidation. Their accumulation in light-grown *Myxococcus* is proportional to that of the photosensitizer (202). Dark-grown cells in late-stationary phase have an accumulation of protoporphyrin IX and no accumulation of photoprotective pigments. On exposure of these cells to light, photoexcited oxygen could increase to levels greater than that used in carotenoid synthesis, with a resultant diversion to the competing photoprocess, which leads to lysis (204).

Carotenoids and Photosensitization of Animals

In 1964 Mathews showed that β-carotene would afford protection to mice that had been lethally sensitized to visible light by injections of hematoporphyrin (783). Human beings who accumulate porphyrins in the condition known as erythropoietic porphyria (EPP) are extremely sensitive to sunlight. In a trial treatment of 53 patients suffering with EPP β-carotene was found by Mathews and her associates to markedly increase the tolerance of 49 of the patients to sunlight and to artificial light (791).

Cellular Locations of Carotenoids and Endogenous Sensitizers

The cellular location of carotenoids appears to be about the cytoplasmic membrane (787, 789, 1015). After induction, the newly synthesized carotenoids in *M. marinum* seem to be elaborated or deposited on preformed membrane (782, 784). It appears that several sites in the electron transport chain of *Sarcina* membranes (malate dehydrogenase, succinoxidase, and the terminal oxidase) are sensitive to the photodynamic action of toluidine blue. As has been pointed out by Prebble and Huda (938), an exogenous sensitizer such as toluidine blue is of general distribution in the cell. It might be expected that the endogenous sensitizer(s) responsible for the effects of light on carotenoid-less mutants would be of limited distribution in the cell and localized in the neighborhood of concentrations of carotenoids. The dangers to the nonphotosynthetic cell from products of endogenous sensitizers at light intensities used in photodynamic dye experiments are negligible but, with increasing light intensities, killing does occur (788).

The action spectra of mycobacteria suggest that, in some of them, the endogenous photosensitizer might be related to a prophyrin and, in others, to a flavin. Batra has stressed the differences in the action spectra of *Mycobacterium* sp. 1312 and *M. marinum* (106). (This raises the possibility that action spectra might be useful for examining interrelationships among mycobacteria.) For example, Batra and Rilling (110) found *M. marinum* to exhibit maxima at 404, 493, and 577 nm, indicative of a porphyrin-like action spectrum, in contrast to the updated maxima found by Howes and Batra for *Mycobacterium* sp. 1312, in which peaks are found at 280 to 285, 365 to 370, 443 to 448, and 465 to 470 nm, suggestive of a flavin-like action spectrum (524). In the case of *M. xanthus*, protoporphyrin IX was associated in early studies with the endogenous sensitizer (202, 204) (see Protection Against Photoinduced Cell Death). Circumstantial evidence has suggested that singlet oxygen may be critical to the lethal photooxidation that activates the endogenous sensitizer, and this has been discussed in some detail by Krinsky (664). In a recent comparative study of photodynamic oxidation and radiofrequency-discharged $^1O_2$ oxidation of guanine, Kornhauser et al. (645) have suggested that, whereas two oxidation mechanisms appear to operate in the photodynamic dye system, only one of these is operative in the excited-sensitizer-oxygen-produced singlet oxygen reactions.

Mathews-Roth et al. have expressed the opinion that the general correlation between protection against light and efficiency of $^1O_2$ quenching by the carotenoids of *S. lutea* argues for mediation by singlet oxygen of light-induced damage in that organism (792). However, their examination of a mutant strain producing a carotenoid with only eight conjugated double bonds has led them to conclude that, besides quenching ability, other factors such as concentration and location of pigment in relation to photosensitizer and to sensitive cellular site may well be important in determining the protectiveness of a given carotenoid pigment (792). Recently Prebble et al. (939) have suggested

that an object of photodamage in *S. lutea* is the respiratory quinone, menaquinone. Menaquinones are important respiratory molecules in mycobacteria and other members of the CMN group (96).

Sensitivity of Mycobacteria to Ultraviolet Irradiation

Tsukamura has considered the danger of UV light to mycobacteria (1178). In a series of six strains of mycobacteria (two constitutively chromogenic, two inducible but not induced, and two nonchromogenic strains) exposed to UV irradiation, the sonstitutive strains were more resistant, the nonchromogenic strains were somewhat less resistant, and the uninduced inducible strains were the most sensitive. No information on the lysogenic status of these strains was given. The conclusion was reached that carotenoid pigments protect against UV irradiation. David has examined the UV sensitivity of apparently nonpigmented, constitutively pigmented, and photoinducible strains (286). Each was grown in the dark prior to irradiation. Killing curves were obtained in the usual way by determining the number of colony formers as the surviving fraction. A UV-sensitive strain of *Escherichia coli* was included. Taking the sensitivity of the *E. coli* strain as 1.0, the following order of sensitivities was obtained: *M. tuberculosis*, 0.4; *M. fortuitum*, 0.25; *M. avium-intracellulare*, 0.22; *M. marinum*, 0.19; *M. kansasii*, 0.18; *M. smegmatis*, 0.16; *M. flavescens*, 0.11. *M. flavescens* is a constitutively chromogenic strain and exhibited one-tenth the sensitivity of the *E. coli* strain and one-fourth that of *M. tuberculosis*. The lysogenic status of these strains was not given. In general, their radiation response was said to be correlated with the size of their genomes, their cellular content of carotenoids, and their capacity for DNA repair.

The correlation found between the presence of carotenoid pigments in mycobacteria and decreased UV sensitivity agrees with the finding of Morris and Subden (856) that albino mutants of *N. crassa* and colored strains inhibited in carotenoid synthesis by β-ionone showed a greater sensitivity to UV irradiation (LD_{50}, 4.35×10^3 ergs/mm^2) than the wild type (LD_{50}, 7.30×10^3 ergs/mm^2), whereas all strains exhibited similar γ-ray survival curves. The authors point out that, although the carotenoids do not absorb in the UV region, their double-bond system is capable of quenching photosensitized or excited compounds. As a case in point they cite Foote's (387) demonstration that singlet oxygen excited from NaOCl plus H_2O_2 ($NaOCl + H_2O_2 \rightarrow NaCl + H_2O_2 + {}^1O_2$) undergoes quenching in the presence of β-carotene (see also [664] and [793]).

These results regarding UV sensitivities in relation to the presence or absence of carotenoids do not agree with the conclusion of Kunisawa and Stanier (675) that nonpigmented mutants of *Corynebacterium poinsettiae* are killed by UV at the same rate as the pigmented wild type. A similar conclusion was reached by Mathews and Krinsky (785) regarding the killing of a strain of *Sarcina lutea* and its colorless mutant by UV. It is difficult to assess these conclusions, since survival curves were not presented and details of UV irradiation were not mentioned. The latter authors gave survivals at two times of exposure to UV. At an exposure of 3 min, 0.95% of the wild type had survived in contrast to 0.65% of the colorless mutant. At 5 min, 0.32% of the wild type and 0.36% of the mutant had survived.

Evolutionary Implications of Patterns of Carotenoid Biosynthesis

M. phlei has been suggested as an archetypal *Mycobacterium* (1189). If that be true, the occurrence in *M. phlei* of a number of distinctive carotenoids might indicate that, early in the evolutionary tree of mycobacteria, pathways for the biosynthesis of a multiplicity of these pigments existed (505). To what extent has this ability been lost in strains of mycobacteria long associated with the darker recesses of animals? Does the white *M. bovis* synthesize phytoene or phytofluene? Does a lack of carotenoids make it more difficult for such bacteria to survive outside hosts? Does the fact that *M. tuberculosis* forms yellow growth on aged bovine serum (1266, p. 519) indicate the possibility that, provided certain intermediates, it could synthesize carotene(s), or is serum only affecting the amount of flavin produced? *M. kansasii* appears in man, at least, not to be a contagious organism. It has been isolated rarely from dust (Jeffries, as reported in [667]) and perhaps more commonly from tap water (82, 199, 773). Does its photoinduced hypersynthesis of carotenoids aid in its survival in a terrestrial or lacustrine environment? Would a constitutive mutant of *M. kansasii* (scotochromogen), because of the fact that its carotenoid synthesis is always turned on, have more chance of surviving in sunlight?

[*Note:* 2-(*p*-diethylaminoethoxybenzal)-*p*-methoxyacetophenone has been described as a derepressor of carotenoid biosynthesis in a wide variety of plants, in *Phycomyces*, and in *Rhodospirillum rubrum* with resultant 10- to 15-

fold yields of pigment over normal (797). This would seem a useful tool for reexamining inducible and noninducible strains of chromogenic mycobacteria.]

THE MYCOBACTERIAL CELL WALL: THE MUREIN-ARABINOGALACTAN-MYCOLATE

The cell walls of mycobacteria consist of a basal structure of murein (= peptidoglycan = muropeptide) covalently linked to an arabinogalactan-mycolate (32, 272, 577, 578, 704). The basal murein of the CMN group (71, 73, 96) has repeating units of β-1,4-linked pyranosides of *N*-acetylglucosamine (GlucNAc)-*N*-acetylmuramic acid (MurNAc) tied in to L-alanine (L-Ala)-D-glutamic acid-NH_2 (D-Glu-NH_2)-*meso*-diaminopimelic acid-NH_2 (*meso*-DAP-NH_2)-D-alanine (D-Ala). Notable is the amidation of the *meso*-DAP (590, 651, 922, 1193, 1257) at the terminal carboxyl end and the participation of D-Glu as an *isoglutamyl* grouping (see Fig. 19). In *Mycobacterium*, as much as 70% of the cross-linking in the murein exists as interpeptide bridges between *meso*-DAP residues (274, 1256). This use of DAP for cross-linking is reflected in the lower alanine content found in mycobacterial cell walls (657, 1256, 1258). In the walls of *M. smegmatis* ATCC 21732 the cross-linking involves D-alanyl-(D)-*meso*-DAP and *meso*-DAP linkages in a ratio of 2:1. An additional distinguishing feature of mycobacterial murein is that most of its muramic acid is *N*-glycolyl-muramic acid (MurNGl) rather than MurNAc (8, 68, 73, 75, 921). A unit of mycobacterial muramyl peptide is shown in Fig. 19. (This unit, linked to arabinogalactan mycolate [Fig. 20], becomes the mycolic acid-arabino-galactan-muramyl peptide "monomer" [see section on Ultimate Mycobacterial Adjuvant].) The rigid polymeric layers of murein, as they appear in ultrathin sections of various mycobacteria, may be found marked *Mur* in electron micrographs comprising Fig. 5B, 8, and 10B (see also Fig. 4). Quite early, the presence of lysozyme-sensitive MurNAc-GlucNAc linkages (246) in these peptidoglycans were demonstrated by Takeya et al. (*M. phlei*, 1132) and by DeWijs and Jollés (*M. fortuitum* and *M. kansasii*, 297). Among other enzymes that have been of use in analyzing mycobacterial mureins are the *Chalaropsis endo-N*-acetylmuramidase and the *Streptomyces albus* G DD-carboxypeptidase (430, 1256). The value of cell wall chemistry in the taxonomy of bacteria has been magnificently presented by Ghuysen (430) and Schleifer and Kandler (1030). The mycobacterial wall in relation to waxes and other complex lipids has been spelled out by Lederer (704).

The polysaccharide which is common to the

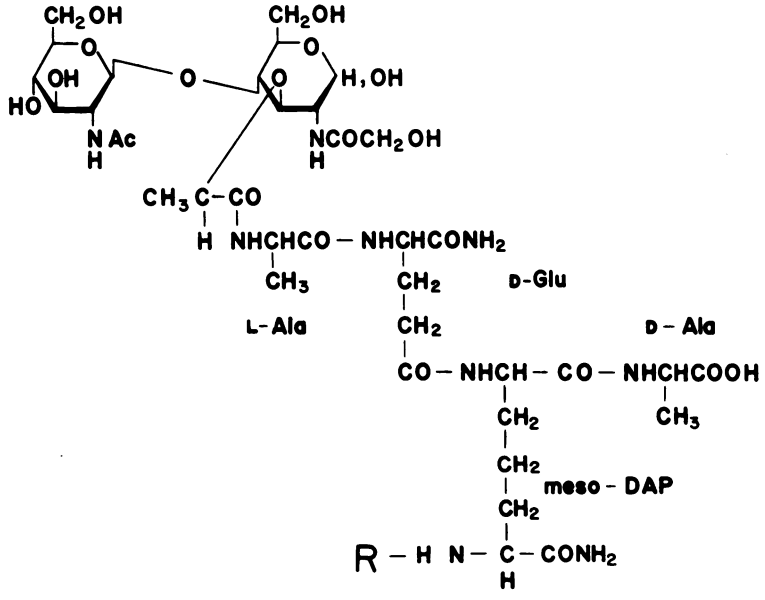


FIG. 19. *Mycobacterial mureinic (peptidoglycan) unit, consisting of N-acetylglucosamine, muramic acid, L-alanine, D-glutamic acid, meso-diaminopimelic acid, and D-alanine. R = another unit beginning with an interpeptide bridge between the meso-DAP (shown) and a second meso-DAP linked in turn to D-Glu, L-Ala, etc. See also Fig. 20 and sections on Murein-Arabinogalactan and Ultimate Mycobacterial Adjuvant.*

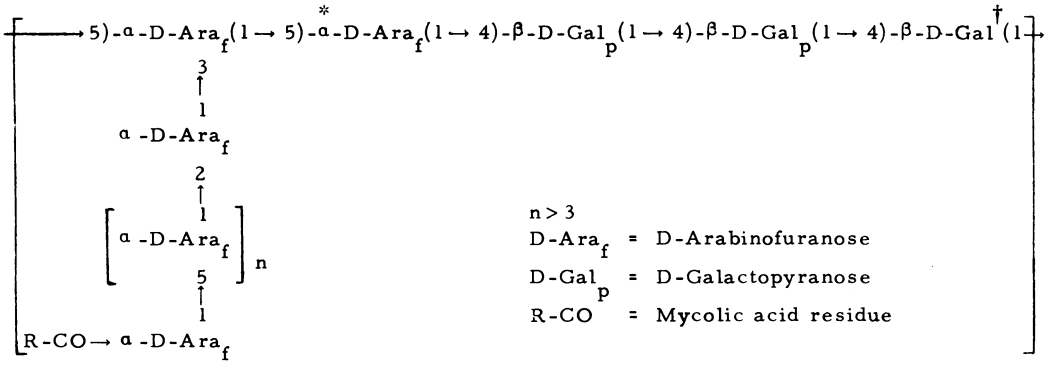


FIG. 20. *Possible structure of the mycobacterial arabinogalactan unit, adapted from Misaki et al. (826). Kanetsuna (578) has suggested that the arabinogalactan mycolate may be tied into the muramyl peptide at the * (upper left), sometimes by phosphate bridges, sometimes via GlucNAc and MurNAc. Several mycobacterial arabinogalactan preparations examined by Misaki et al. interacted with antisera prepared against cell walls of Corynebacterium diphtheriae and Nocardia asteroides. The close similarity of the arabinogalactans of the CMN group has been further examined by Azuma et al. (68). See section on Arabinogalactans in text. For further information on biological activity of arabinogalactans, see Birnbaum and Affronti in section on Soluble Antigens and section on Delayed Hypersensitivity and Fig. 19. † Vilkas et al. (1204) have obtained a digalactoside from the arabinogalactan of cell walls and wax D of M. tuberculosis identified as 6-O-β-D-galactofuranosyl-D-galactose. The presence of D-galactofuranosyl units in the arabinogalactan suggests that for those saccharides a 1 → 4 linkage between arabinofuranose and galactofuranose does not obtain. Misaki et al. point out that such units "may originate from . . . secondary galactosidic linkages."*

cell walls of the CMN group is an arabinogalactan. In *Mycobacterium* it consists of 5 arabinose to 2 galactose units linked as 1 → 5-D-arabinofuranose and 1 → 4-D-galactopyranose (32, 33, 77, 826, 827) (for the presence of 6-*O*-β-D-galactofuranosyl-D-galactose, see Fig. 20). This arabinogalactan polymer links the murein of the inner wall to the mycolic acids of the outer envelope (Fig. 19).

Arabinogalactan and Arabinogalactan-Mycolate

The α-branched, β-hydroxylated long-chain fatty acids of the CMN group, R—$\overset{\beta}{\underset{\text{OH}}{\text{CH}}}$—$\overset{\alpha}{\underset{\text{R}_1}{\text{CH}}}$—COOH, discovered by Anderson (40, 41) and elucidated by Asselineau (59), Asselineau and Lederer (62), Lederer and Pudles (706), Pudles and Lederer (948), and Etemadi et al. (357) are among the hallmarks of the CMN group. They range in carbon numbers from C_{28} to C_{40} (corynomycolic acids) to C_{40} to C_{60} (nocardic acids) to C_{60} to C_{90} (mycolic acids). Although some of the shorter-chain mycolic acids, such as this $C_{32}H_{64}O_3$ corynomycolic acid, CH_3—$(CH_2)_{14}$—$\underset{\text{OH}}{\text{CH}}$—$\underset{\text{C}_{14}\text{H}_{29}}{\text{CH}}$—COOH, are made by *C. diphtheriae* and *C. ovis* (298, 706, 949), those genera that synthesize longer-chain mycolic acids are *also capable of producing some corynomycolic acids*. The mycolic acids are found as mixtures of homologues. From species to species there are wide variations in these structures resulting from differences in the lengths of their chains and the localization of groupings of ketone, hydroxyl, cyclopropane, carboxyl, etc., especially on the main chain. The occurrence of these substituents depends on the biosynthetic capabilities peculiar to different species of mycobacteria. Etémadi has discussed the uniqueness of certain mycolic acids in relation to the taxonomy of mycobacteria and to their phylogeny (352–354) (see Krassilnikov et al. regarding mycolic acids in chromogenic paraffin-oxidizing mycobacteria [663]). Azuma and Yamamura made the valuable observation that mycolates recovered from the bound lipids of walls of *M. tuberculosis* Aoyama B were mycolates of arabinose (74, 76). Since then, ample evidence has accumulated to confirm their suggestion that some mycolic acids of the envelope were linked through their carboxyl groups to the 5-hydroxyl of arabinofuranose units that terminate branches of the arabinogalactan of the cell wall (32, 33, 75, 77, 825, 827).

Continuing the investigations of Misaki et al. (77, 827), Misaki et al. (826) have found the repeating units of the arabinogalactans of *M. bovis* BCG, *M. tuberculosis* H_{37}Rv, H_{37}Ra, and Aoyama B, *M. phlei*, *M. smegmatis*, and *Mycobacterium* sp. P1 to consist of ramified struc-

tures of 11 to 16 sugar residues of α-(1 → 5)-, major, and α-(1 → 2)-, minor, D-arabinofuranoside linkages and β-(1 → 4)-D-galactopyranosidic [or β-(1 → 5)-furanosidic] linkages. The side chains terminate in arabinofuranose residues and are attached to the main chains at C3 of arabinose and perhaps at C6 of the galactose residues (Fig. 20) (see also [33] and [652]). Arabinose mycolates have been further studied by Acharya et al. (4), using mass spectroscopy, and evidence has been obtained for specific structures having carbon numbers ranging from about C_{80} to C_{91}. These were derived from arabinomycolates in the envelopes of strains of BCG and *M. kansasii*. Kanetsuna and San Blas (578) have proposed a structure for the intact murein(peptidoglycan)-arabinogalactan-mycolate from the walls of *M. bovis* BCG and *M. smegmatis* ATCC 14468, where mycolic acids are linked at carboxyl ends to D-arabinofuranose (5-OH) and through them to the arabinogalactan polymer. The arabinogalactan is then tied into the basement peptidoglycan through either (i) GlucNAc, (ii) MurNGl, or (iii) a phosphodiester bridge joining MurNGl. After the finding of muramic acid-6-phosphate in the walls of *Mycobacterium butyricum* by Liu and Gotschlich (731), Kanetsuna demonstrated its presence in the walls of BCG (577); Cunto et al. reported it in the walls of *M. smegmatis* (274), and Kotani et al. gave evidence for its presence in the walls of *M. tuberculosis* $H_{37}Rv$ (657). (For an analysis of the chemical composition of the cell wall of *M. tuberculosis* $H_{37}Ra$, see Acharya and Goldman [3].) Amar and Vilkas have recently reported the isolation of an alkali-stable arabinose phosphate from the walls of $H_{37}Ra$ (31). Thus, bases for the linkages shown in Fig. 20 exist. (Monomycolates of arabinose have been found in fractions from cell walls of *M. tuberculosis* [74], *M. bovis*, and *M. kansasii* [4], and a dimycolate of arabinose [$C_{147}H_{280}O_{10}$] was found in extracts of *M. scrofulaceum* [*marianum*]. One of the mycolic acids is monoethylenic and dicarboxylic; the other has one carboxyl group and two cyclopropane rings. Through their carboxyl ends, they are ester linked at the 3-hydroxyl of arabinose [195].) Arabinofuranosyl side chains of mycobacterial arabinogalactans are, apparently, primarily responsible for the immunological cross-reactions between mycobacterial polysaccharides, per se, and those of *C. diphtheriae* and *N. asteroides* (826).

Isolated and purified arabinogalactan reacts with the jack bean lectin, concanavalin A (Con A), and the interacting sites appear to be at the C2, C3, and C5 hydroxyls of the α-D-arabinofuranosyl residues (438). A survey of the Con A agglutinability of filtrates from several species of mycobacteria indicated that strains of more than one species released arabinogalactan (and, perhaps, some arabinomannan) into the medium, whereas other strains of those same species, as well as certain other species, released no reactive material (279).

Arabinogalactans are of wide distribution in nature. The arabinogalactan peptide from the endosperm of wheat is agglutinated by castor bean lectin, and this agglutination is strongly inhibited by galactose and methyl β-D-galactoside. In the wheat arabinogalactan peptide, the arabinogalactan units are tied via their galactosyl ends into the peptide core through hydroxyproline residues. Most likely, the linkages between the arabinose and galactose are not β-1,4 (374).

Teichoic Acids

Up to now there have been no reports of polyol-phosphate chains, teichoic acids, in the walls of members of the CMN group (our search, plus personal communication from J. Baddiley). In analyses of delipidated cell walls of corynebacteria, mycobacteria, and nocardias carried out in this laboratory by S.B. Arden and B.L. Beaman, plus additional studies by B.L. Beaman elsewhere (personal communication), total cell wall (delipidated) phosphorus of more than 0.1% was not found. This augurs against the presence of teichoic acids in these bacteria.

Early Studies on Fragments of the Mycobacterial Cell Wall

Ether-soluble, acetone-insoluble, chloroform-extractable peptidoglycolipid components derived from old cultures of *M. tuberculosis* were early designated as "waxes" (waxes D: Anderson [40, 41], Asselineau [59], Jollès et al. [555, 556], Aebi et al. [12]).

Asselineau (59, p. 241) has said, "Waxes D of human strains are essentially composed of peptidoglycolipids, i.e. esters of mycolic acids with a polysaccharide containing D-galactose, D-arabinose, and D-mannose and glucosamine linked through galactosamine residues to peptides formed of alanine, glutamic acid, and α-ε-diaminopimelic acid; muramic acid has also been detected," and he cites the detectors, Stewart-Tull and White (1099). This finding of components common to wax D and the rigid layer of the mycobacterial cell wall (Fig. 19) suggested that wax D either was a part of the wall or was material synthesized in excess of that needed for insertion into the wall. Kotani et al. (651) showed that wax D could be got out

of intact cell walls ("bound" wax D) with suitable enzymes. Kanetsuna has discussed the mural location of wax D in relation to various procedures used for its extraction (577). Interference with the synthesis of cell walls in *M. tuberculosis* $H_{37}Rv$ by cycloserine causes the accumulation in the medium of arabinogalactan-galactosamine-DAP-mycolate (289). If one uses "waxes D" as a term to designate parts of the backbone of the mycobacterial cell wall (Fig. 19), then wax D-active or -cross-reacting material could be generated by disordered cell wall synthesis (either genetically or antibiotically induced) singly by specific or in concert by several autolytic enzymes. The accumulation of excess cell wall material during growth, the induction of unfinished cell wall products (precursors) by antibiotics, and the release of cell wall components by enzymatic and acid hydrolysis are well-known events for a number of bacteria (137).

The mycobacterial cell wall is an elaborate structure. A section of it will contain material from each of the three layers shown graphically in Fig. 4 and actually in Fig. 5: mucopeptide-arabinogalactan-mycolate and components which make up L_1 and L_2 of Fig. 4. Depending on the CMN bacterial strains being considered, other polysaccharides will also be found in "waxes": e.g., glucans, mannans, and arabinomannans are found in a range of CMN species including *M. bovis*, *M. smegmatis*, and *M. phlei*, as well as in *C. diphtheriae* and *N. asteroides* (67). Lederer has suggested that bound wax D, liberated by enzymatic hydrolysis, be considered a "monomer" of the cell wall (704). Certainly, as Kanetsuna's work suggested, "wax D" is an early designation for a fragment of the mycobacterial wall that could be extracted in a particular way. Undoubtedly, autolysis occurring in old cultures contributed to its extractability. For recent coverage of other information concerning wax D, see the excellent review by Goren (453).

The adjuvant activity of mycobacterial "waxes" has been much investigated (see section, Adjuvant Action). Stewart-Tull and White (1100) have made the valuable observation that the time of harvesting of mycobacteria markedly affects the yields of adjuvant-active peptidoglycolipids obtainable from them. They used the following protocol: washed cells were extracted with alcohol-ether (1:1) for 30 days. Organisms were then collected on a filter and reextracted with chloroform. Pooled chloroform extracts were concentrated by distillation, and the crude wax obtained was extracted with hot methanol and boiling acetone, repeatedly, until no more material dissolved. The residue was called purified wax D. The aim of the study was to examine the importance of the age of the culture upon the production of waxes D by *M. phlei*, *M. balnei*, *M. fortuitum*, and *M. smegmatis*, with control studies of *M. avium*, *M. kansasii*, *M. tuberculosis*, and *M. tuberculosis* BCG. The more rapidly growing organisms (e.g., *M. phlei* and *M. smegmatis*), when harvested at 7 days, yielded markedly more peptidoglycolipid than at 28 days. Although the peptidoglycolipid from young cultures possessed adjuvant activity, the predominantly glycolipid extract from old cultures did not. The authors point out that the hydrosoluble moiety of the peptidoglycolipid (missing from the glycolipid extracted from old cultures) contains those amino acids, hexosamines, and sugars found in the murein of the mycobacterial cell wall and they suggest that, in the absence of nutrient in the culture medium, mycobacterial growth is maintained by autonutrition (45) from peptidoglycolipid. Their results make it clear that all *naturally occurring* mycobacteria, whether they are called "saprophytic" or "parasitic," produce adjuvant-active peptidoglycolipids.

Beyond the Arabinogalactan-Mycolate, "Nonpeptidoglycan" Amino Acids, and Surface Peptidoglycolipid

Sohler et al. (1074) noted an absence of proline- and sulfur-containing amino acids from cell walls of *N. rubra* 3639, in which they found more than trace amounts of arginine, aspartic acid, glycine, lysine, serine, threonine, valine, phenylalanine, leucine, and isoleucine. Snyder et al. (1073) confirmed the presence of minor amounts of several amino acids in thoroughly washed and unextracted cell walls of nocardias. Cummins (271, 273) and Yamaguchi (1290) observed that alkaline ethanol removed these "background amino acids." Some investigators had suggested that the amino acids found in small quantities in cell wall hydrolysates were the result of contamination with cytoplasmic components. Beaman, by carrying out comparative, quantitative analyses of (i) cell walls digested with trypsin and pepsin, (ii) cell walls digested with trypsin and pepsin and extracted with alkaline ethanol, and (iii) whole cells extracted with alkaline ethanol, prior to rendering them into cell wall preparations and treatment as in (i), has demonstrated rather conclusively that these nonpeptidoglycan amino acids are associated with the lipoidal component of the surface of the nocardial cell (113). (It is interesting in this respect that Misaki et al. [828], using entirely different methods, found aspartic acid, valine, leucine, isoleucine, and

serine associated with the murein of BCG prior to treatment with Pronase.) Beaman has since extended his findings to *Corynebacterium* and *Mycobacterium* and related the amino acids (as peptides) and lipids (peptidolipids) to patterns found on the surfaces of members of the CMN group (112). A catalog of electron micrographs of these patterns of the L_2 outer envelopes of mycobacteria, with, for reference, two corynebacterial and two nocardial species, comprises Fig. 22 to 26.

Wietzerbin-Falszpan et al. (1258) coined the term "nonpeptidoglycan" amino acids and have attributed to them 15% of the weight of crude delipidated BCG cell walls. About half of these amino acids could be removed with hot dodecyl sulfate, suggesting that they were from lipoproteins or glycolipoproteins noncovalently linked to the arabinogalactan-mycolate-peptidoglycan. The fraction of material not removed by hot dodecyl sulfate treatment may represent material that is covalently linked to one of the basal structures. The nonpeptidoglycan amino acids, then, to a great extent go to make up peptidolipids and peptidoglycolipids of the outer envelope, which will be considered categorically in the following sections.

As shown in Fig. 4, the outer lipoidal material of the mycobacterial cell wall can be resolved into two layers (L_1 and L_2-L_3). Figures 5 and 21 show the outermost lipoidal coating of *Mycobacterium* sp. NQ and *M. lepraemurium*, respectively. This material has been called a capsule of mycoside C by Draper and Rees (315). In Figures 22 through 26 are shown the L_2-L_3 patterns of reference strains of the CMN group.

Ropelike Patterns: the Surface or Subsurface Glycolipids, Peptidoglycolipids, and Peptidolipids

The nonpeptidoglycan amino acids just discussed are associated with the lipoidal surface of mycobacteria. Exactly how, and as parts of what kinds of molecular units, is far from completely known. The ropelike structures responsible for the markings shown on the various negatively stained mycobacteria in Fig. 22 to 24 have been said to be of glycolipid or peptidoglycolipid composition (113, 445, 530, 1018, 1131, 1132, 1134, 1135).

Visualization of the subsurface of the glycolipids of members of the CMN group can be accomplished by the technique of negative staining (see also 313). This material assumes a ropelike appearance in mycobacteria, as is shown in Fig. 22 to 24. Sometimes, demonstrat-

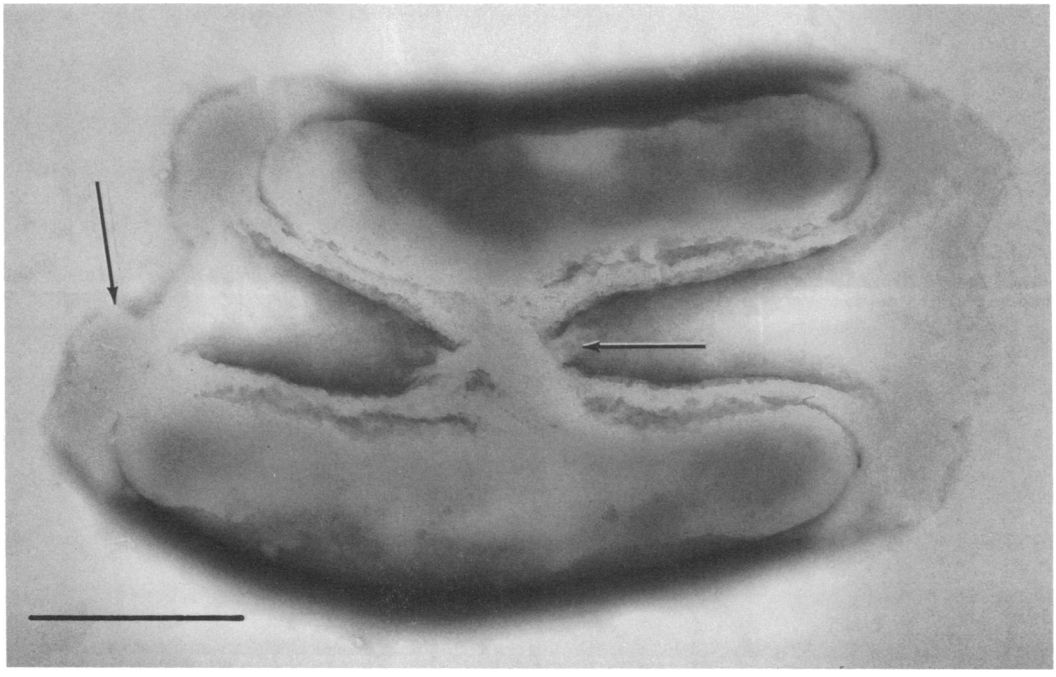


FIG. 21. *Freshly grown cells of M. lepraemurium were prepared for electron microscopy after being once washed with phosphate buffer (pH 7.2). Note two interconnected organisms completely surrounded by nonstaining substances (arrows). Note also sonicated cells for the comparison (Fig. 22A). (×28,000; bar = 1 μm.) See section on Mycobacteria Growing In Vivo and In Vitro. See Tables 1, 2, and 3.*

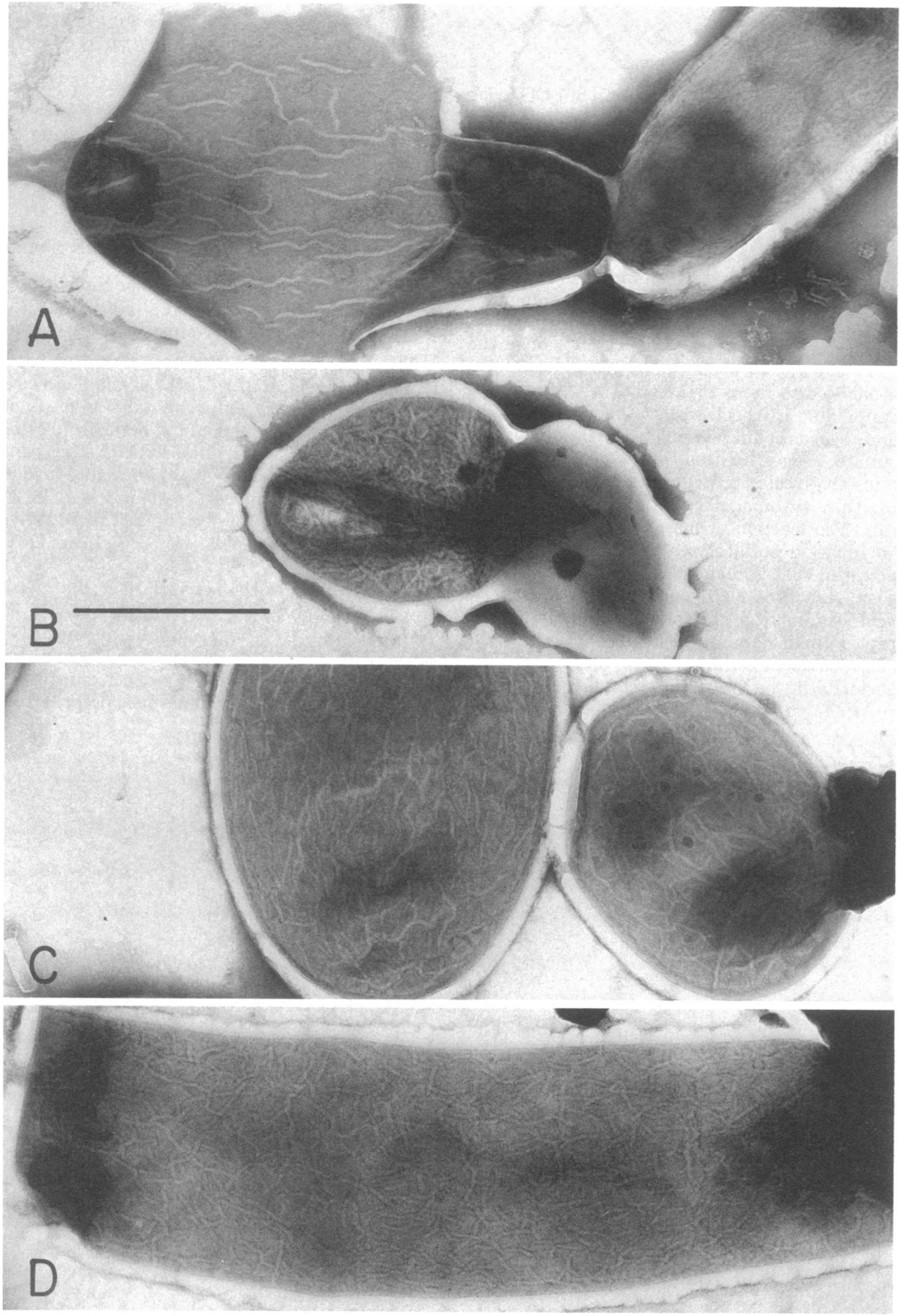


FIG. 22. *Ramified fibrous structures from representative mycobacterial species revealed by negative staining. See also legend to Fig. 6, where the extent of this wrinkled material appears to vary from species to species. A large sampling shows wide variation in the amount of ropelike material among the cells of any given species. (A) M. lepraemurium, (B) M. thermoresistibile, (C) Mycobacterium sp. 1285, and (D) M. kansasii. (×60,000; bar = 0.5 μm.) See Tables 1, 2, and 3.*

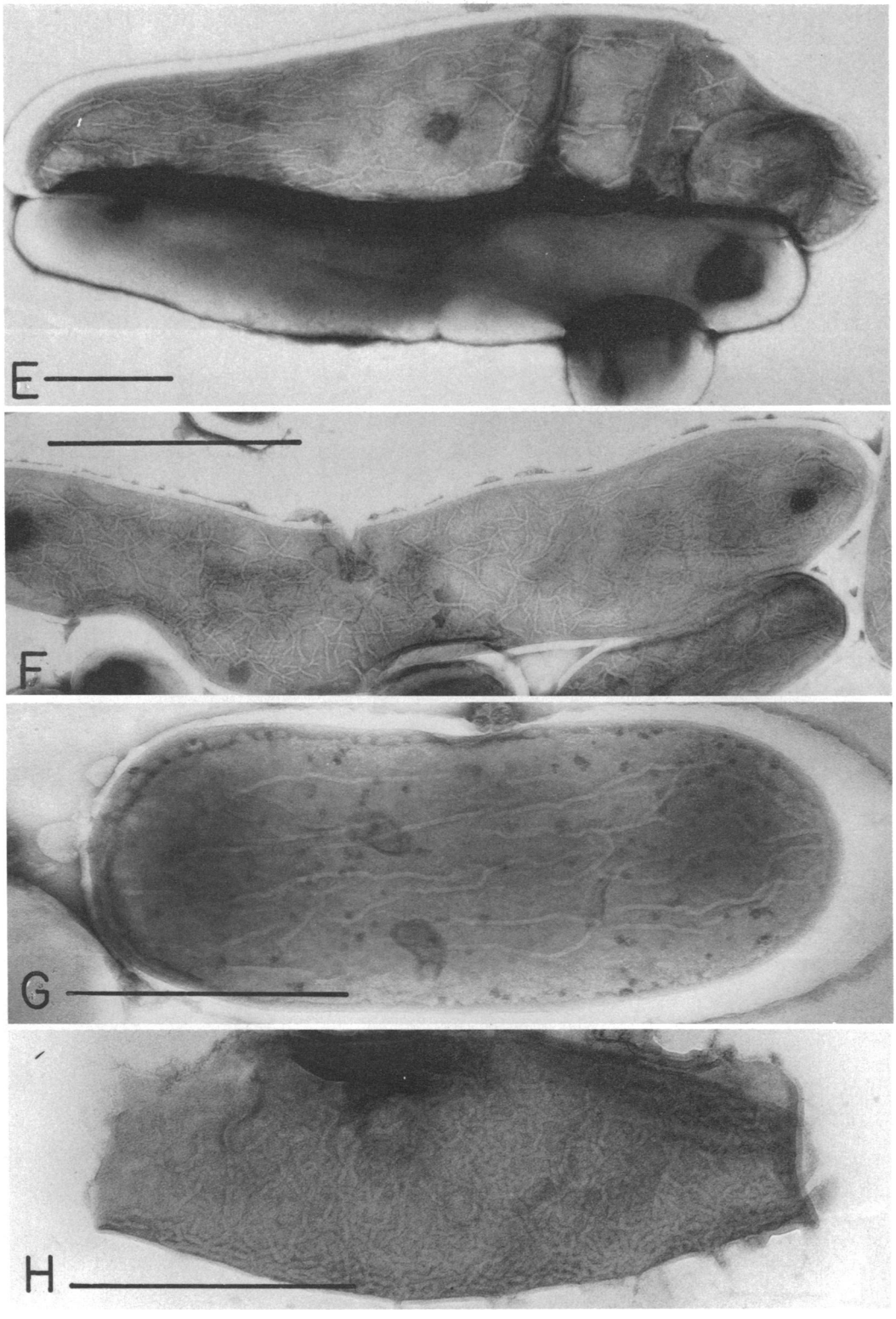


FIG. 23. *(E) M. tuberculosis $H_{37}Rv$ 102 (×40,000), (F) M. smegmatis 607 (×78,000), (G) Mycobacterium sp. I (×86,000), (H) M. ulcerans (×88,000). (All bars = 0.5 μm.) See also Tables 1, 2, and 3.*

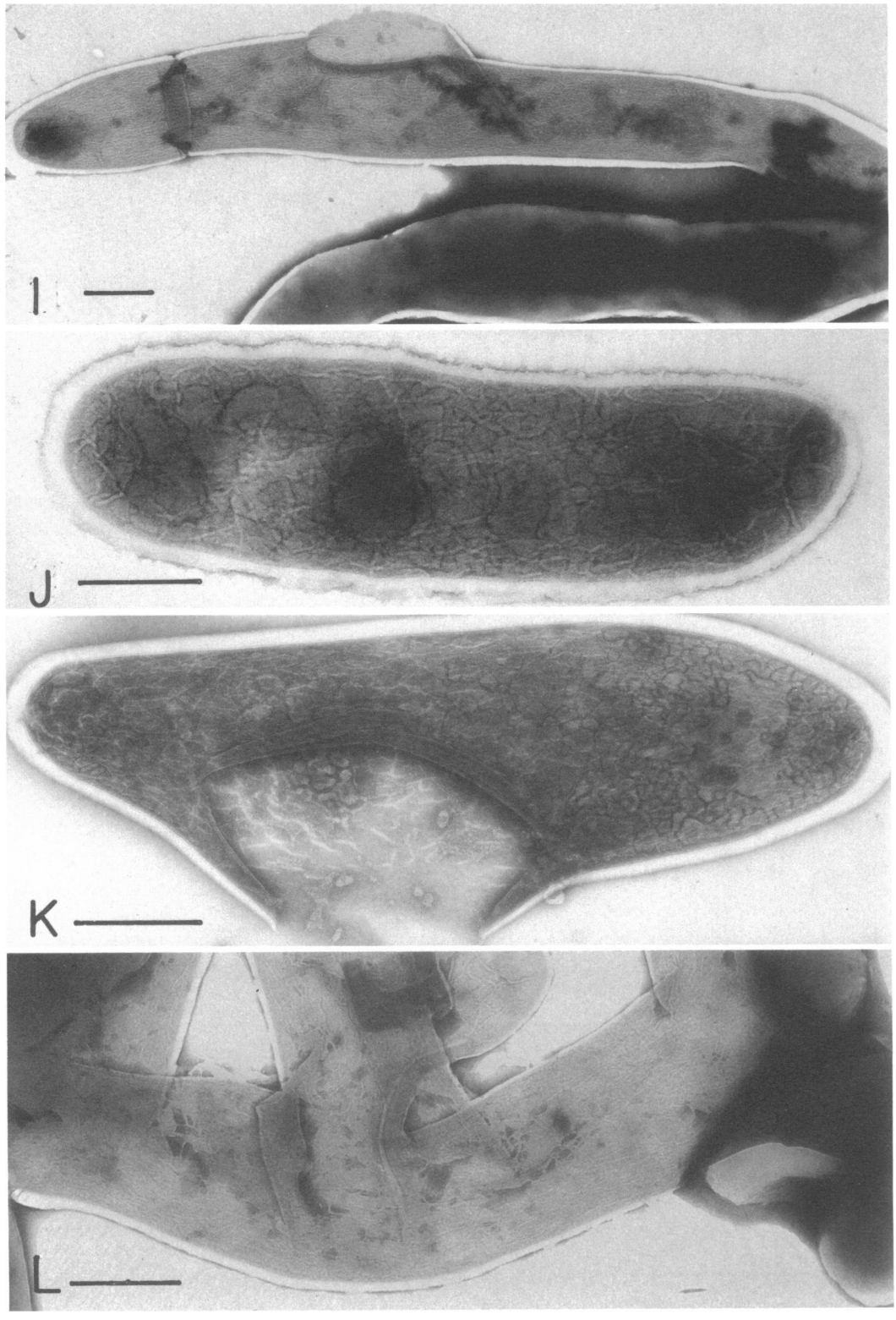


FIG. 24. *(I) Mycobacterium sp. NQ (×21,000), (J) M. phlei (×39,000), (K) Mycobacterium sp. 1582 (×39,000), (L) M. intracellulare (×34,000). (All bars = 0.5 μm.) See also Tables 1, 2, and 3.*

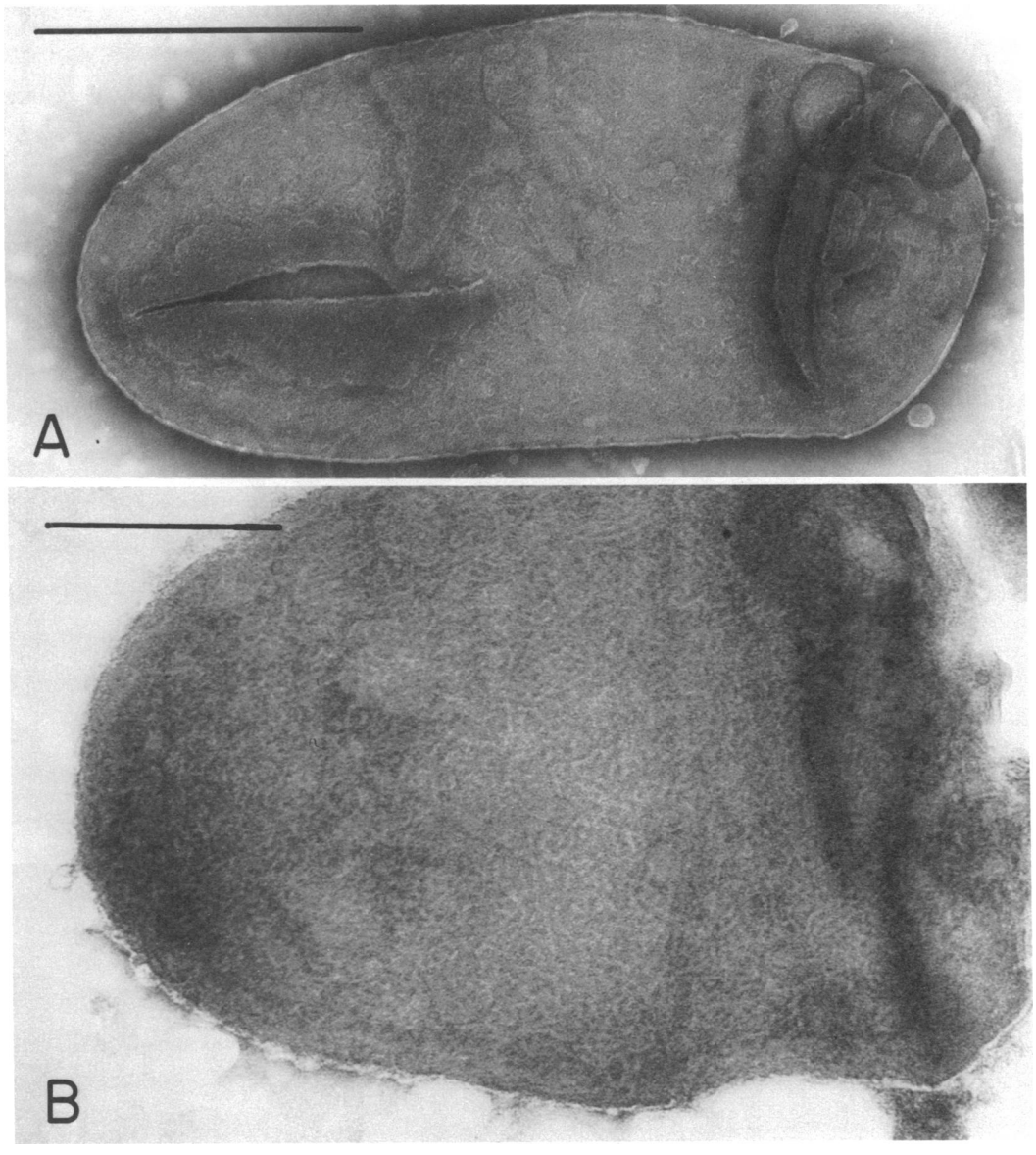


FIG. 25. *Two corynebacterial species exhibiting different fibrous patterns. (A) C. diphtheriae $C7_s$, with ruptured cell wall showing small vessicular and short fibrous structures. Compare with Nocardia and Mycobacterium. (×88,000; bar = 0.5 μm.) (B) Corynebacterium sp., Medalle X. Wrinkles (ramified structures) of the wall are more pronounced than in C. diphtheriae (above) and more compact than those seen in either Nocardia or Mycobacterium. (×125,000; bar = 0.25 μm.)*

ing the ropes is made difficult by a material that covers them up (see L_1 in Fig. 4 and 5 A). In such cases, subjecting the cells to sonic vibration tends to make the ropes more distinct. An examination of the ropes of cells at various stages of growth leads to the conclusion that they represent folds of glycolipid or peptidoglycolipid that perhaps are more stretched out in actively growing cells and more wrinkled in post-log-phase cells. It is obvious, from our discussions concerning the lipoidal bodies that become evident in aged cells and from those data showing that carotenoid synthesis is maximal in stationary-phase cells, that the biochemistry of the nondividing mycobacterial cell is distinctive. A type picture of the ropelike structures of *M. smegmatis* 607 comprises Fig. 6. An examination of the ropelike structures of *M. leprae-*

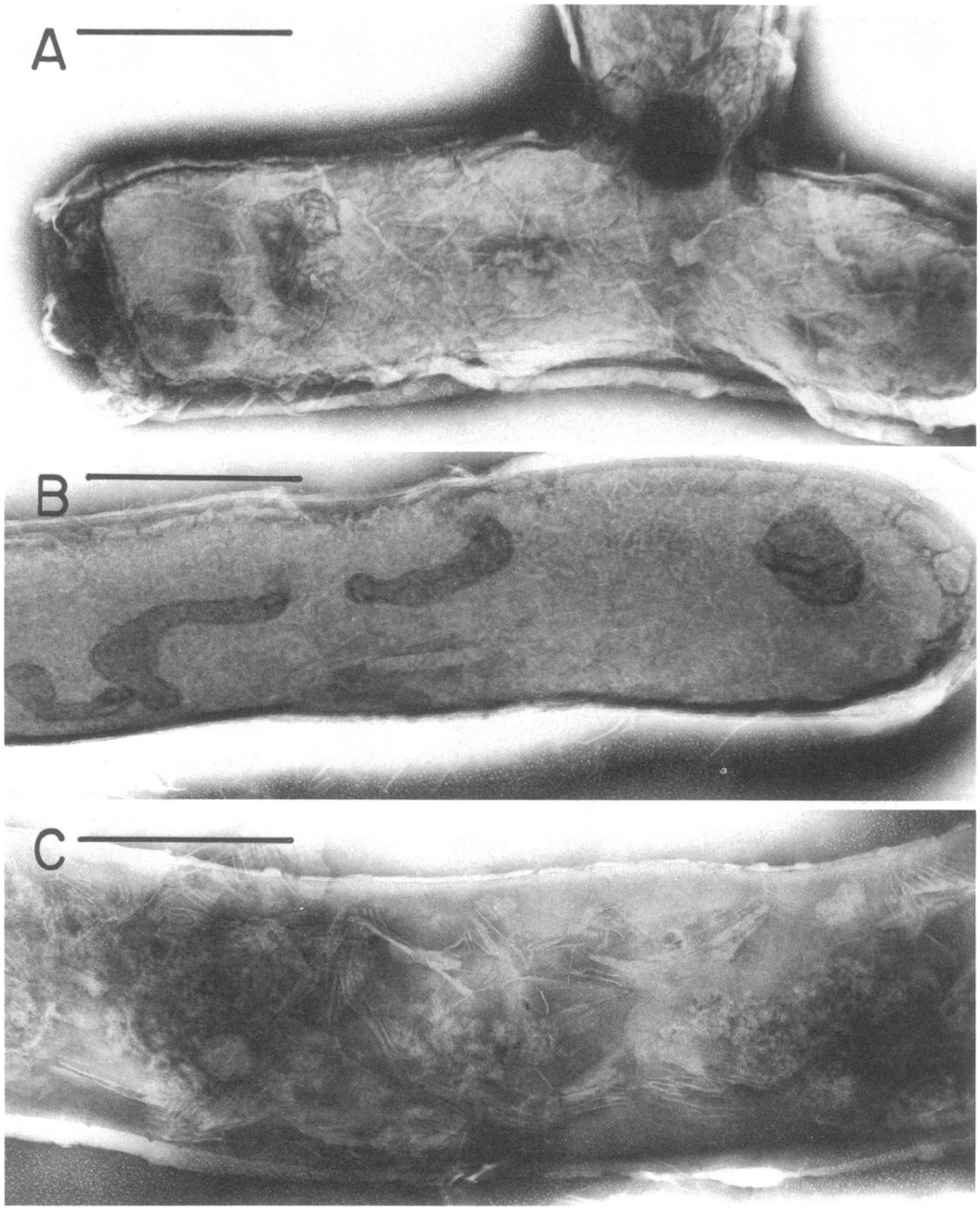


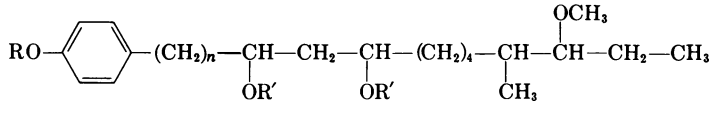
FIG. 26. *Negatively stained cells of two strains of Nocardia asteroides showing the organization of surface fibrous materials. The ramified fibrous configurations are smaller (2 to 8 nm in diameter) than those of mycobacteria and offer a comparatively irregular pattern. (A) N. asteroides 92. Note irregular mass of material from the outer wall and smaller fibrous structures; also, metachromatic granule at the point of branching. (×150,000; bar = 0.2 μm.) (B) N. asteroides 133. Compare this fine ramified pattern with those of mycobacteria. (×150,000; bar = 0.2 μm.) (C) N. asteroides 133. Fibrous pattern is obscured by sheathlike (peptidoglycolipid) material that lies external to it. To a lesser degree, this obscuring is evident in Fig. 26B. See text under peptidoglycolipid for a discussion of outer sheathlike materials. (×150,000; bar = 0.2 μm.)*

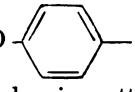
murium (Fig. 22A), *M. thermoresistibile* (Fig. 22B), *Mycobacterium* sp. 1285 (Fig. 22C), *M. kansasii* (Fig. 22D), *M. tuberculosis* $H_{37}Rv$ (Fig. 23E), *M. smegmatis* 607 (Fig. 23F), *Mycobacterium* sp. ICRC (Fig. 23G), *M. ulcerans* (Fig. 23H), *Mycobacterium* sp. NQ (Fig. 24I), *M. phlei* (Fig. 24J), *Mycobacterium* sp. 1582 (Fig. 24K), and *M. intracellulare* (Fig. 24L) indicates the range of patterns and dimensions found in these foldings. Similar surface structures of *C. diphtheriae* C7, *Corynebacterium* sp., Medalle X, and *N. asteroides* 92 and 133 are to be found in Fig. 25 and 26, respectively. Specific sequences comprising the peptidolipids and peptidoglycolipids of mycobacteria are discussed in the following section.

Glycolipids, Peptidoglycolipids (Mycosides), and Peptidolipids of the Outer Envelope

Over the past two decades there has been recovered from mycobacteria a series of glycolipids and peptidoglycolipids; in many cases, these have in common terminal saccharide moieties containing rhamnoses that are *O*-methylated in various positions. These compounds are named mycosides, and they were reported to be species specific (1066, 1067, 1068). Lederer (702) has thoroughly reviewed the literature of mycosides up to 1967 (see also Goren [453]), pointing out that they fall into two general categories.

Category I. Here are included the mycosides that are lipidic aglycones, consisting of glycosides of *p*-phenols having a branched glycolic chain whose hydroxyls are esterified with straight- and branched-chain fatty acids. Gastambide-Odier and Sarda (423), employing special methods of oxidation, have established the structures of the ethers of the phenol-glycols of mycoside A (from *M. kansasii*) and mycoside B (from *M. bovis*) through a combination of paper and gas chromatography and mass spectrography. Their interesting paper contains supportive data for concluding that these two mycosides have the following common backbone:



where R' = the acyl substituents, palmitic and mycocerosic acids. In the case of the mycoside A of *M. kansasii*, R = a trisaccharide of 2-*O*-methylfucose, 2-*O*-methylrhamnose, and 2,4-di-*O*-methylrhamnose; n = 16, 17, 18, 19, 20. (Subsequently, 3-*O*-methylrhamnose and 3-*O*-methylfucose have also been reported from this mycoside [424].) Mycoside B from *M. bovis* is simpler: R = 2-*O*-methylrhamnose and n = 14, 15, *16*, 17, 18. (Mycosides G and G' from, for example, *M. marinum* also fall into this category [420, 1207].) Earlier, it was thought that the phenol of these lipid aglycones originated from tyrosine (702). The authors discuss the origin of the phenolic group mostly in relation to incorporation of label from sodium [3-^{14}C]propionate, and they suggest that the phenol originates via shikimate. Goren et al. (458) have examined certain lipids of a group of strains of *M. tuberculosis* from East Africa, Burma, and the region of South India around Madras. This same group of bacteria had been studied by Mitchison and his associates for their relative virulence (831) and for their general differences from other strains of *M. tuberculosis*, including their sensitivity to H_2O_2, their catalase activity, and their isoniazide sensitivity (832, 833). In extracts of a group of these *M. tuberculosis* strains, which Mitchison had found to be of lowered virulence for the *guinea pig*, Goren and his associates were able to identify a phenolic phthioceryl diester indistinguishable from the phenolic *O*-methyl ether of the aglycone moiety of mycosides A and B. This methyl ether, CH_3O-  , occurs naturally in the strains showing attenuated virulence for the guinea pig. In these mutants, the sugars (2-*O*-methylfucose, 2-*O*-methylrhamnose, 2,4-di-*O*-methylrhamnose, in the case of mycoside A, and 2-*O*-methylrhamnose in the case of mycoside B) have been substituted by an *O*-methyl ether.

Category II. The second group of mycosides is made up of peptidoglycolipids consisting of a sugar moiety, a short peptide, and FA, as shown in Fig. 27. Mycoside C_1, isolated from *M. avium* and characterized by Jollés et al., was of this type (554). Structurally distinct mycosides of category II have been described for *Mycobacterium* sp. 1217 (688), *Mycobacterium farcinogenes* (*Nocardia farcinica*) (689), and other mycobacterial species (59, 453). The mycoside C_2, synthesized by *M. avium*, has been shown by Voiland et al. (1209) to be linked on a pentapeptide of D-phenylalanine-D-allothreonine-D-Ala-

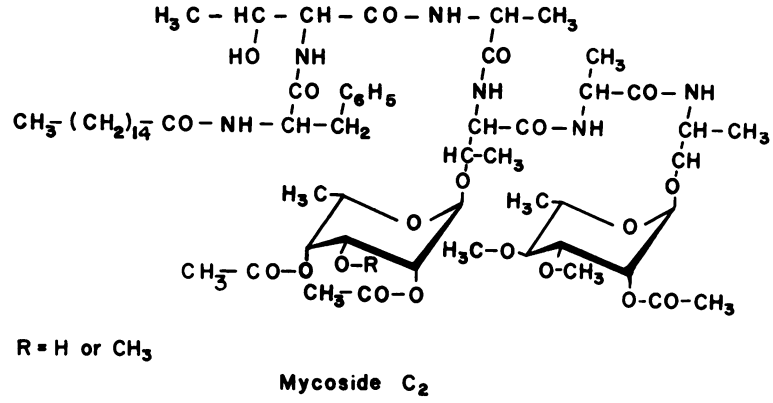


FIG. 27. *Structure proposed by Voiland et al. for mycoside C_2 from Mycobacterium avium (1209), unusual in that the pentapeptide portion of a molecule of this class is the aminoalcohol L-alaninol to which is linked 3,4-di-O-methyl-L-rhamnose. The sugar terminal to allothreonine is 6-deoxytalose or its 3-O-methyl derivative. The lipid moiety of this mycoside consists of a complex mixture of fatty acids containing palmitic acid. Additional described mycosides of category II fall into two subclasses. (A) The peptidoglycolipids of this subclass are comprised of D-phenylalanine-D-allothreonine-D-alanine-L-alaninol conjugated to one or more of the following sugar moieties as indicated: C' = di- or mono-O-methyl rhamnose in M. farcinogenes (689); C_{b1} = di-O-acetyl-6-deoxytalose and tri-O-methyl rhamnose in M. smegmatis (butyricum) (1206); C_s = di-O-acetyl-6-deoxytalose and 3,4-di-O-methyl rhamnose in M. scrofulaceum (1205); C_{1217} = di-O-acetyl-6-deoxytalose, tri-O-methyl rhamnose, and 3,4-di-O-methyl rhamnose, trace amounts, in Mycobacterium sp. 1217 (688). (B) The peptidoglycolipids of this subclass are comprised of D-phenylalanine-[D-allothreonine-D-alanine]$_2$ conjugated to the following sugar moieties: C_1 = di-O-acetyl-6-deoxytalose, 3-O-methyl-6-deoxytalose, and 3,4-di-O-methyl rhamnose in M. avium 802 (554); and of D-phenylalanine-[D-allothreonine-D-alanine]$_3$ conjugated to the following sugar moieties: C_m = di-O-acetyl-6-deoxytalose and 3,4-di-O-methyl rhamnose in M. scrofulaceum (marianum) (237). Note: It is unfortunate that reference materials for most of these compounds are no longer available (personal communications from E. Lederer, G. Michel, M. Gastambide-Odier, and others). See sections on Mycosides, on Tuberculins and Other Mycobacterial Elicitins, and on Mycobacteriophage Receptors. See also Tables 1, 2, and 3.*

D-allothreonine-D-Ala-L-alaninol. To the aminoalcohol, alaninol, is linked 3,4-di-*O*-methyl-L-rhamnose. To the second molecule of threonine is linked 6-deoxy-L-talose or 3-*O*-methyl-deoxy-L-talose. The complex mixture of FAs, including palmitic acid, are linked to phenylalanine. This proposed structure is shown in Fig. 27. In this paper we are concerned with the function and cellular location of various species of macromolecules derived from mycobacteria. It is assumed that the peptidoglycolipids occupy a superficial position in the mycobacterial cell. In the electron micrographs of *Mycobacterium* sp. NQ in Fig. 5, surface structures are clearly shown.

Fourteen years ago, Fregnan and Smith (395) and Randall (396) attempted to relate the presence and absence of mycosides to colonial mutants (cells exhibiting different surface properties from that of the wild or ancestral type and therefore piling up to form identifiably different colonies). They attributed "type specificity" to the mycosides. Recently, support for the surface location of mycoside C has come from studies by Furuchi and Tokunaga (412) using bacteriophage D4 and the mycoside C of the D4-sensitive *M. smegmatis*, termed mycoside C_{sm}. This component in isolated form blocked the adsorption of phage to the bacterium by which it was synthesized. *M. smegmatis* D4, a mutant resistant to D4, failed to adsorb D4 and failed to synthesize mycoside C_{sm}. Goren and associates have independently reached similar conclusions concerning D4 and mycosides C_{1217} and $C_{scrofulaceum}$ and have published beautiful pictures of D4 phages fixed onto filamentous needles of C mycosides derived from *M. smegmatis* and *Mycobacterium* sp. 1217 (459). These studies leave no doubt that the receptors for D4 are in the mycoside and, therefore, indicate that in D4-adsorbing strains of *Mycobacterium* the mycoside is at the surface. Bar mutants that are resistant to D4 may be of two kinds: (i) those that no longer synthesize receptors for D4 and (ii) those that elaborate, at their surfaces, substances that cover up the phage receptors (10). Some years ago, Lanéelle and Asselineau, in discussing the possible function of mycoside C_{1217}, with its polar portions and its nonpolar portions, suggested that mycosides might be located at the periphery of the mycobacterial cell (688; see also [315]). It now seems that

mycoside C_{1217} *is* located peripherally but its function remains to be discovered. Serving as a phage receptor is not fulfilling a microbial function. Very likely, these mycobacterial lipid-saccharide-peptide moieties serve for inward transport in a wet environment and, in conjunction with mycolates, sulfolipids, etc., serve to prevent loss of water from the cell during periods of excessive drying. The advantage they give to some cells in invading host animals may be similar to that provided more virulent strains of *Salmonella typhimurium* by specific terminal sugars on their O-antigenic side chains. For example, *S. typhimurium* serotype 1, 4, 12, which has the O antigen lipopolysaccharide side chains terminate in the dideoxyhexose, tyvelose, is more virulent for mice than *S. typhimurium* 1, 9, 12, which has, instead of tyvelose, the dideoxyhexose, *abequose*, as the terminal sugar (765).

Category III. Peptidolipids. If the surface antigens of mycobacteria generally consist of peptidolipids with saccharide moieties or other substituents, one would expect to find strains (mutants) lacking one or more of the capacities needed to introduce the sugar moieties into the peptidoglycolipids. Species of *Mycobacterium* are known which produce only peptidolipids (687a, 689a). In the case of mycoside C, for example, Lanéelle has the impression that bacteria which produce this mycoside do not produce detectable lipopeptides, and mycobacteria which produce lipopeptides do not seem to yield mycosides (personal communication). An investigation of the genetics of mycoside synthesis may well furnish us with information on the biosynthesis of these compounds. They would seem in many ways to be analogous to the lipopolysaccharides of *E. coli* where the elucidation of the regions I (lipopolysaccharide), II (core), and III (lipid A) stemmed from concerted biochemical and genetic efforts (97).

Other Molecular Species Associated with the Mycobacterial Cell Surface: Phosphatidylinositol Oligomannosides

We have suggested that dikes of phosphatidylinositol oligomannoside may extend from under the peptidoglycan layer to the surface of the cell. Evidence for or against this notion, which originated with Kotani (653), could be obtained by haptenic inhibition and other serological studies.

MYCOBACTERIA GROWING IN VIVO (Phe I) AND IN VITRO (Phe II)

The adaptive processes of microbes would lead one to expect in vivo-grown organisms to differ to some degree from those grown in vitro. The matter of differences existing between in vivo- versus in vitro-grown mycobacteria has been under consideration for some time (see Table 1 in reference 973). The pinpointing of differences between populations of $H_{37}Rv$ grown in vivo (in the lungs of mice) and populations grown in vitro was begun by Segal and Bloch (1040), who demonstrated that the in vivo phenotype (Phe I) and the in vitro phenotype (Phe II) of $H_{37}Rv$ (shown to be qualitatively comparable as to dry weight and viable count) differed significantly in their capacities to reduce 2,3,5-triphenyltetrazolium chloride in a Tween-albumin medium with 0.5% glucose and in the extent of the stimulation of their mean Q_{O_2} values over endogenous rates by glucose, glycerol, lactate, acetate, pyruvate, benzoate, benzaldehyde, salicylate, *n*-heptanoate, octanoate, and oleate. The endogenous rates of respiration for Phe I and Phe II were similar. Phe II was 2.7 times as active as Phe I in hydrogen transfer and showed a marked increase in Q_{O_2} with 11 of 11 added energy sources, whereas Phe I was stimulated by only 4 of 11. These four (see above) were salicylate and the three FAs. Both phenotypes were stimulated by extracts from infected mouse lungs but not by extracts from normal lungs. Cultures (in vitro) of $H_{37}Rv$ derived from Phe I behaved in all respects like those of $H_{37}Rv$ (Phe II). Thus, there are marked differences in the two phenotypes and the differences must involve barring of some of the energy molecules from Phe I.

In a subsequent paper (1041), Segal and Bloch recounted a visible difference between suspensions of phenotypes I and II. The mouse-grown cells showed far less tendency to form large clumps, yet they were less able to pass through filter paper (Whatman no. 12, fluted) than in vitro-grown cells. Phe II, as a phenol-killed vaccine, was a better immunogen than Phe I, but Phe I proved to be more virulent for mice than Phe II. Segal later extended these studies of the immunogenicity of the two phenotypes by examining (i) protection against infection (in CF_1, 6-week-old, female mice), (ii) capacity to *elicit* DH (in randomly bred, albino, tuberculous guinea pigs), and (iii) capacity to *induce* DH (in white New Zealand rabbits). Whereas phenol-killed vaccines had previously been used, heat-killed cells (with and without IFA) and sonically disrupted cells were employed as three separate means of immunization. In the mouse protection experiments, as well as in the elicitation and induction of DH, Phe II was a better immunogen than Phe I. Segal has stated that various preparations of Phe I appear to exhibit the same biological

properties whether derived from one passage or 25 serial passages through mice (1038). Since the shift Phe I ⇌ Phe II is readily reversible, it is obvious that the genome of $H_{37}Rv$ (the wolf) remains the same, though properties related to transport are temporarily modified (sheep's clothing). The fact that Phe I lacks the neutral red-binding capacity (528, 817) of Phe II certainly suggests a real modification in the surface of the former (1039). Further, Kanai has found that Phe I is resistant to 4% NaOH at 37°C for 4 h, whereas the viability of Phe II is lowered 2 logs by such treatment (571, 572, 573). The most reasonable working hypothesis is that the Phe I bacilli are coated so that the surface features associated with Phe II are unavailable to react with neutral red. Segal and Miller obtained evidence for a quantitative difference between the lipids of Phe I and Phe II (1042). Kondo and Kanai have demonstrated that Phe I and Phe II of the Ravenel strain of *M. bovis* behave with regard to DH and mouse protection much as do their homologues of $H_{37}Rv$ (634, 637). They further provided evidence that Phe I contains cord factor, a *surface* component of Phe II. Kondo et al. (638) showed that 10-methylstearic acid (tuberculostearic acid) was present in Phe I of *M. bovis*. Kanai et al. (575) demonstrated the presence of phthiocerol dimycocerosate and mycolic acids in Phe I of *M. bovis* Ravenel. The surface component, mycoside B (see section on Peptidolipids), was not found in extracts of Phe I bacilli of the Ravenel strain.

The presence of Phe I of $H_{37}Rv$ within J-111 cells (derived from leukocytes of a case of human leukemia) did not prevent cell division or cause vacuolation as long as the numbers of bacilli were small (2 to 10/cell). When the numbers of bacilli per cell were large, excessive cording was evident. If cord factor were a major contributor to death of the cells, then that from intracellular bacilli must have been responsible for death. Experiments were carried out with added cord factor and with numerous bacilli cording *extracellularly*. Under these conditions, the J-111 cells remained healthy. This finding led the authors to conclude that extracellular cord factor was tolerated by the cells under study (190).

Rabbits infected with *M. bovis*, *M. avium*, and *Pasteurella pseudotuberculosis* show elevated levels of serum cholesterol, phospholipid, and triglyceride (1151a). Experiments using [2-^{14}C]mevalonic acid suggest that mechanisms for the disposal of serum cholesterol are impaired in infected (*M. bovis*) rabbits (1151b). Among the host's lipids that are closely associated with harvested bacilli of Phe I are esters of cholesterol (636, 638). Unsaturated octadecanoic acids account for most of the esterification (637). Kondo and Kanai carried out a series of lipid analyses on casein-induced (and uninduced) harvested macrophages and demonstrated that the induced macrophages contained a higher proportion of phospholipid and cholesterol ester though a lower level of total lipid than the uninduced cells (635). Thus, there is probably available to the macrophage-engulfed mycobacterium a large amount of cholesterol. Bacteria in general, when in the presence of cholesterol, take it up (e.g., the uptake of [4-^{14}C]cholesterol by washed *Streptobacillus moniliformis* was 17,000 cpm/mg of cell protein, whereas its L-form took up 19,820 cpm/mg, *M. phlei* took up 125 cpm/mg, and *C. diphtheriae* took up 1,725 cpm/mg [961]). Is the difference between Phe I and Phe II a layer acquired in the host? Is the layer primarily lipoidal? Does the layer contain some protein, some antibody? The report that agglutinins active against *M. tuberculosis* have a ubiquitous distribution among human beings (93) would seem to make it inevitable that Phe I tubercle bacilli would attach some such antibody to their surfaces.

Brown et al. (191) have pointed out that obligately parasitic *M. lepraemurium* is surrounded by an electron-transparent zone (ETZ), which separates the bacterium from the cytoplasmic components of rat fibroblasts in which they grow. They have suggested that this zone offers protection from the host (see also [314]). They equate these areas with those seen in sections of granulomas induced in hamsters with heat-killed $H_{37}Rv$ (325) and, presumably, with those surrounding living intraphagosomal *M. microti* (495). There is no doubt that, for a large number of bacteria, there exists an outer transparent zone (OTZ of reference 394). The OTZs found in most figures in this review are probably equivalent to L_1 (and L_2, to a limited extent) shown in Fig. 4 and 5. In sections of mycobacteria within animal cells, however, the exaggerated OTZ of Brown et al. seems to be a result of fixation and embedding effects upon one biological entity within another. This is rather obvious in the hamster pictures (325) of intracellular $H_{37}Rv$. Thus, one probably cannot render visible the differences between Phe I and Phe II. The data accumulated by Kanai (571, 572) and Kanai and Kondo (574) do enable us to attribute some of the differences between Phe I and Phe II to the result of an intimate association of Phe I with macromolecules of the host. Kanai (571, 572) showed that in vivo-grown $H_{37}Rv$ and BCG had associated with them murine acid phosphatase (MAP). When living in vitro-grown bacilli were exposed to

MAP, they bound it, and its presence seemed to block the activity of mycobacterial acid phosphatase. The accumulation of enough host macromolecules about the surface of Phe I should render it somewhat indistinguishable from the host's self.

Most Phe I appear to have an intact cell wall. This is interesting in light of the fact that Willet and Thacore found that either acid phosphatase or phospholipase C could render $H_{37}Ra$ susceptible to lysozyme, with subsequent protoplast formation (1263) and, with high enough numbers of bacteria, even L-form development (1262). The addition of lysozyme to cell cultures of J-111 human monocytes has made possible the formation of protoplasts in these host cells (1147). Perhaps host agents act to destroy these wall-less types of mycobacteria? For an interestingly documented discussion of the plasticity of in vivo *M. tuberculosis*, see N. A. Schmelev on "Polymorphism of Mycobacteria" (1031). See also the section Autolysis, Protoplasts, and Mycobacterial L-Forms. Salient features of Phe I and Phe II are shown in Table 5. Examples of aging Phe I comprise Fig. 12.

GRANULOMAS

The Tubercle and Granulomatagenesis

Presumably, one of the goals of the last 100 years of mycobacterial research has been to understand the pathogenesis of the chronic granulomatous disease, tuberculosis. The chronicity of the granulomatous diseases seems directly related to the capacity of the infecting microorganisms (protozoa, fungi, bacteria, and others) to survive in the macrophages of the host. Robbins (985, p. 335) has given the following textbook description of a tuberculous granuloma: "the center of the microscopic granuloma is occupied by a small nest or aggregate of plump, rounded mononuclear cells that vaguely resemble epithelial cells and are therefore designated as epithelioid cells. . . . The epithelioid cells are thought to be mononuclear phagocytes that have ingested intact or fragmented tubercle bacilli. In the margin of this cluster of epithelioid cells and sometimes within the center of the cluster, there are large, multinucleate giant cells. . . . About this cluster of epithelioid cells and accompanying giant cells, there is a peripheral zone or collar of plump fibroblasts and lymphocytes." This is the *hard tubercle*. Subsequent necrosis of the central zone, *caseation necrosis*, provides what the pathologist regards as the most *characteristic feature of the tubercle*. The biology of the granulomatous inflammatory exudate has been clearly presented by Spector (1078).

TABLE 5. *A few of the known differences between in vivo and in vitro phenotypes of Mycobacterium tuberculosis $H_{37}Rv$*[a]

Phe I = in vivo[b]	Phe II = in vitro
Q_{O_2} elevated by 4/11[c]	Q_{O_2} elevated by 11/11
Stick together when harvested	Not so sticky
5× more virulent for mice	5× less virulent for mice
Poor immunogen	Good immunogen
Does not bind netural red	Binds neutral red
Bound cholesterol and antibody	No bound cholesterol; no bound antibody
Murine MAP[d]	None: but will bind

[a] Probably any mycobacterium causing a chronic infection in any given animal must be able to exist as an in vivo phenotype in the macrophages of that animal (see also Collins [255]).

[b] See text. Note that the Phe I of *M. bovis* Ravenel has been shown by Kondo and Kanai to produce tuberculoprotein in the lungs of mice. This product moves like PPD in disc gel electrophoresis and has good tuberculin activity in skin tests (634).

[c] For substrates used, see text.

[d] MAP, Murine acid phosphatase.

For extensive discussions of the development of tuberculous granulomas, see Rich (973) and Lurie (740). Over the last decade Lurie and Dannenberg and Dannenberg and his associates have used a variety of techniques for studying tuberculosis in rabbits and for examining cellular reactions in tuberculous (BCG, $H_{37}Rv$) infections (741, 1052). The incubation in vitro of excised dermal and pulmonary tuberculous lesions with tritiated thymidine ([^{3}H]T) under conditions of oxygenation provided material for quick freezing and cryostatic manipulation and sectioning. Autoradiography, staining for β-galactosidase (an inducible enzyme used by the authors as an indication of the activated state of a macrophage), staining for acid-fastness, and counterstaining with hematoxylin prepared the experimental material for assessment (741). The authors found that macrophages undergoing epithelioidization still incorporated [^{3}H]T. "Fully mature" epithelioid cells were rich in β-galactosidase (4+) but showed no [^{3}H]T incorporation. The authors stated that "tubercle bacilli did not stimulate macrophage(s)" to divide. In fact, bacilli-laden macrophages, which by the criteria of the authors were activated, instead of dividing, died, taking their place in the caseous centers. Thus, cell division seemed not to be the way these macrophages reduced their bacillary load. It was the authors' impression that dividing macrophages were those recently arrived (in the lesion) from the bloodstream, unsurfeited and hungry. These authors subsequently (44, 281) found that in the first 2 weeks of development of dermal (rabbit) BCG-induced lesions, mononuclear cells entered at a rate roughly in pro-

portion to growth (increase in size) of the lesion. Lesions of reinfection developed faster and, presumably, migration into them was at an increased rate. As previously, the authors concluded that most of the divisions of mononuclear cells in the lesions occurred early after arrival. Since lesions in reinforced animals showed an accelerated development, the authors suggested that DH influences the overall process "by increasing a) the number of mononuclear cells entering the tuberculous lesions, b) their local rate of division, c) their death rate and d) their rate of activation" (44). In these papers the dynamics of the developing tubercle has been acknowledged. The assumption that the gorged macrophage is a dying one might be tested further. For, although these macrophages do not incorporate [^{3}H]T, it has not been established that they could not be stimulated to do so in the presence of adequate levels of lymphokines. (Despite the established dogma that mature macrophages do not divide [1202], there is good evidence to the contrary [217, 874].) It is not known whether intracellular phenotypes of mycobacteria liberate mitogenic materials that reach the extracellular *milieu*. One would guess that they do not (see discussion of In Vivo and In Vitro Phenotypes). It would be worthwhile to know whether or not the injection of purified protein derivative (PPD) into the kinds of lesions studied by Dannenberg and his coworkers would influence nucleic acid biosynthesis in those cells. If the answer were no, the moribund state of gorged macrophages destined for the "caseous center" would be more firmly established. Although Ando (43) has examined cytologically the tuberculin response in second-infection animals, he offers no information concerning the gorged macrophage. He does, however, point out that, in rabbits with reinfection lesions (animals with specific PPD-associated DH), there is an increased activation of young mononuclear cells in (skin test) areas injected with PPD.

The Bentonite Granuloma

A number of immunologists have taken the position that granulomas formed in chronic infectious processes probably do not relate directly to hypersensitivity of the delayed type. Uhr, in 1966, noted that "the relationship between delayed hypersensitivity and the formation of granulomata is not known" (1190). With regard to establishing such a relationship, Epstein has indicated that "the most urgent problem is to devise suitable laboratory models of granulomatous hypersensitivity. In the past the main stumbling block has been separating colloidal foreign body reactions and allergic granulomas. At this time it seems wise to extend information about the organized epithelioid cell granuloma as a prototype of granulomatous hypersensitivity" (349). Three years later, Reid clearly and succinctly brought the matter into more recent focus, proposing that "the immunologic granuloma is largely a cell mediated reaction occurring under certain particular physicochemical conditions" (966). The experiments of Boros and Warren (161) bear out this statement and spell out most of the suggested "physicochemical conditions." These are conditions that relate directly to the tubercle (see above), the Mitsuda reaction (834), and the clearance of mycobacteria in the competency for clearing bacilli test of Convit and associates (261).

Bentonite (a colloidal aluminum silicate) particles are capable of inducing in experimental animals granulomas of the foreign body type (FBG). Boros and Warren (161) have produced in the lungs of mice (Swiss albino CF1 and C3H/HeJ) FBGs with bentonite. Further, they have employed bentonite to which had been adsorbed soluble antigens derived from *Schistosoma mansoni*, *M. tuberculosis* $H_{37}Ra$, and *Histoplasma capsulatum* for eliciting specific hypersensitivity-type granulomas (HG) in animals infected with the very organisms from which these antigens were derived. The same antigen-coated particles induced *only FBGs in animals carrying a heterologous infection*. The authors also showed that, in infected animals receiving naked bentonite (no adsorbed antigen), only FBGs developed. Moreover, they demonstrated that *infectious granulomatous reactivity could be transferred with immune lymphoid cells* but *not with serum*. They were able to demonstrate that infectious-type granulomas (HGs) would develop around either naked bentonite particles or particles carrying heterologous antigens when *antigen-adjuvant-sensitized* or *infected animals received systemic injections of homologous antigen* along with the particles. The occurrence of HG correlated well with the elicitation by homologous antigens of a delayed swelling in the footpads of the sensitized animals.

Dunsford et al. (326) have used both bentonite and latex (styrene divinyl benzene copolymer) particles in conjunction with soluble schistosoma egg antigen (SEA) for examining granuloma formation in relation to DH in the mouse. Although the latex was a particle better suited to histological procedures, bentonite proved superior in that it absorbed more antigen. In the footpads of sensitized mice, SEA

elicited an early acute inflammatory response followed by DH dermal response lasting 2 to 4 days. SEA adsorbed onto bentonite or latex particles elicited a histologically typical granulomatous response in the sensitized mouse lung. The peak of the reaction was between 2 and 4 days and lasted 16 days for bentonite and 8 days for latex. Peritoneal macrophages from the sensitized mice were blocked in their migration after addition of SEA. These experiments show that the response of the sensitized animal to diffuse and particle-sequestered antigen is different and, in essence, the difference is the manner in which the antigen has been presented (diffuse versus sequestered). The authors found that, although most of the antigen was released from particles within 60 min, immunofluorescence indicated that it persisted in lung granulomas for at least 24 h.

Experimental Granulomatous Systems

Galindo and Myrvik (416) made the observation that rabbits sensitized subcutaneously with heat-killed BCG-in-oil emulsion failed to develop pulmonary granulomas but did develop dermal sensitivity to tuberculin. Alveolar macrophages procured from such animals were not consistently inhibited by PPD in the migration inhibition test. On the other hand, the majority of animals sensitized with the same 100-μg dose of BCG given intravenously responded with the formation of pulmonary granulomas with increased ratios of lung weight to body weight and increased populations of alveolar cells. Although the majority of such animals did not develop dermal sensitivity to tuberculin, alveolar cells procured from those showing an extensive granulomatous response were inhibited by PPD in a migration inhibition test.

Giant Cell Formation In Vitro

Galindo has exploited the pulmonary granulomatous response of rabbits toward gaining some insight into the requirements for giant cell formation (see above). A strong pulmonary granulomatous response could be elicited in these animals 4 weeks after the intravenous administration of $H_{37}Ra$ suspended in mineral oil. Harvested alveolar cells from such rabbits, following 12-h incubation with heat-killed $H_{37}Ra$, fused to form multinucleated giant cells (30 to 700 nuclei per cell). Products such as (i) heat-killed *E. coli*, (ii) heat-killed *B. subtilis*, (iii) latex particles, ovalbumin, or phytohemagglutinin failed to stimulate fusion and giant cell formation. The addition of immune serum enhanced the formation of giant cells, and supernatant fluids from alveolar cells of sensitized animals incubated with $H_{37}Ra$ provoked giant cell formation by alveolar cells from nonsensitized animals (414). More recently, Galindo et al. (415) found that cell-free fluids from BCG-sensitized lymph node cells (6×10^6/ml), incubated with 5 μg of heat-killed BCG per ml, induced extensive giant cell formation among alveolar macrophages from nonimmunized (normal) rabbits. Although neither *E. coli* nor *B. subtilis* could serve as an inducing antigen in this system, *Nocardia braziliensis* could elicit the production of macrophage fusion factor in the same lymphoid cells (from BCG-immunized animals). It would be worthwhile to know whether the elicitation of fusion factor is possible with just any member of the CMN group.

Giant Cells in Beryllium Granulomas

Beryllium granulomas develop after the injection of beryllium (BeO) into sensitized animals. Sensitivity to beryllium is transferable by cells, and there is a good correlation between the formation of migration inhibition factor (MIF), lymphocytotoxin, and sensitivity as measured by skin tests. The production, in response to PPD, of MIF by cells from tuberculin-negative berylliosis patients under steroid treatment remains an unresolved phenomenon worthy of further investigation (779). Black and Epstein (138) tested the ability of giant cells, in epithelioid granulomas induced with zirconium and beryllium salts, to incorporate [^{3}H]T. They interpreted the failure of the nuclei of these cells to show tritium labeling 40 min after exposure to mean that "normal nuclear division" does not occur in giant cells. They postulated that epithelioid cells containing vesicles developed "in damaged and necrotic areas, and that mainly this type of epithelioid cell fuses to form giant cells."

Cord Factor Granulomas

Bekierkunst et al. (116) have reported that intravenous injection into mice of "amounts of trehalose-6,6-dimycolate as small as 1 to 5 μg . . . induce[s] in the lungs of mice the formation of tubercles in which the cellular composition is indistinguishable from that in tubercles formed after infection with living BCG bacilli." Obviously, the authors in this statement did not regard populations of *M. bovis* BCG (absent from these aggregates) as cells, nor did they consider empty macrophages different from macrophages that had engulfed BCG. Further, they made no distinction between foreign body-type granulomas and infectious agent granulomas (see results of Moore et al.,

just following). These authors also reported an increased protection of cord factor-treated mice against challenge with $H_{37}Rv$. More recently, Bekierkunst and Yarkoni have stated that "Cord factor in the form of an emulsion is unable to sensitize mice to react with a more extensive granulomatous response to a subsequent challenge with the same substance" (119). They have reported that mice infected with BCG become sensitized to cord factor and that this DH is different from that elicitable with PPD. Further, whereas PPD administered to BCG-infected mice did not elicit a granulomatous response, the administration of cord factor to such mice resulted in extensive granuloma formation.

Moore et al. (850) have used a quantitative migration inhibition test for investigating the role of cord factor in tuberculous granulomatagenesis. Their migration unit was equal to the number of 5-mm^2 areas occupied by cells migrating in a chamber over a 30-h period at 37°C. The migration index = (migration units of cells in presence of Agn)/(migration units of cells without Agn) × 100. The animals were injected according to protocols that yielded comparative data on the capacities of 100 μg of trehalose-6,6′-dimycolate (oil-in-water emulsion) and of equivalent amounts of killed BCG (i) to elicit *chronic* granuloma formation after intravenous injection, as well as pulmonary DH, (ii) to elicit pulmonary DH in rabbits sensitized with killed BCG, and (iii) to sensitize rabbits to undergo *acute* granulomatous response upon challenge (at 3 weeks) with whole (5 mg/ml of 0.15 M NaCl) BCG. Although lungs from the cord factor-sensitized animals contained numerous microscopic granulomas, the volume of packed cells (macrophages) that could be collected from the lungs was scarcely more than was found in normal lungs and about 1 to 2% of the packed volume of cells collected from lungs of BCG-sensitized rabbits. The results of the migration inhibition test for DH in cells from cord factor-injected rabbits were nil. The capacity of cord factor to function in the migration inhibition test employing cells from BCG-sensitized rabbits was nil in the face of significant inhibition by PPD and BCG. Animals sensitized with killed BCG responded to challenge with whole BCG by an *acute* granulomatous response. Animals sensitized with cord factor did not produce a response detectable by the methods used.

Are FBG and HG Poles Apart?

Spector (1078) has applicably termed the granuloma an *outpost* of the reticuloendothelial system enjoying "the constant renewal and reinforcement of similar cell collections elsewhere" as, for example, the spleen or bone marrow. Following on the heels of the diapedetic polymorphonuclear leukocytes (PMNs) at a time no earlier than 4 h, the mononuclear infiltration begins. The emigration of these cells (mostly from the bone marrow) is followed by some cell transformation and some cell division. The sustaining of the infiltrate is ascribed to (i) longevity of some constituent cells, (ii) recruitment of fresh mononuclear cells, and (iii) mitotic division of macrophages. The nature of the overall reaction found in granulomas initiated by beryllium or zirconium (in humans), bentonite, carrageenan or IFA, on the one hand, and *B. pertussis*, CFA, BCG, etc., on the other, are different. The former, the *foreign body-type granuloma* (FBG), and the latter, *the epithelioid* or the *hypersensitivity granuloma* (HG) (see Epstein [349]), are seemingly poles apart. In FBG, there is a low turnover of the mononuclear phagocytes and they show little tendency to divide. Spector feels that maintenance of such lesions is due in large part to the longevity of the macrophages, and he suggests that this may be a special property of those macrophages peculiar to FBG. The HG lesion shows a reduction in the bacterial load, constant arrival of new macrophages, the development of giant cells and epithelioid cells, and the accumulation of large numbers of lymphocytes. Variations in these two polar types may occur as the result of additional reactants contributed by the host animal: e.g., the development of antigen-antibody complexes within the lesion, the coating of the bacterial cell with some soluble self component acquired from the macrophage (see Glynn [433]; see section, Mycobacteria Growing In Vivo and In Vitro), etc. These are modifications that tend to shift the response from HG towards FBG. Convit (261) has provided experimental proof for such a modified HG in lepromatous leprosy. A supportive finding for the clinical diagnosis of lepromatous leprosy, the most disfiguring form of the disease, is a negative lepromin reaction. Lepromin is an autoclaved preparation of human leprosy bacilli contaminated with a "minimal amount" of human tissue. Whereas persons with lepromatous leprosy (by definition) give a negative response to lepromin, persons suffering from tuberculoid leprosy respond to the injection of lepromin by giving an HG response. Convit has shown that, whereas the lepromatous patient forms an FBG in response to lepromin, that patient gives an HG response to each of several other mycobacteria. The factor responsible for this peculiar response to human leprosy bacilli, as opposed to other mycobacteria, has not yet been found. It might be an

antibody to a human antigen; it might result from a very singular idiosyncrasy with regard to antigen recognition, etc. It certainly is not the result of general anergy (1184) or general immunosuppression (1036).

The fact that macrophages from cases of lepromatous leprosy give an HG reaction to a variety of mycobacteria but an FBG reaction to lepromin would seem to mitigate against the idea (1078) that the macrophages found in HG are qualitatively different from the macrophages found in FBG.

MYCOBACTERIUM AS ANTIGEN

Agglutination

Although mycobacteria are effective in stimulating overall immune responses, serological analyses of them have lagged far behind those done on other bacteria. Their hydrophobic nature (1019) and the inagglutinability of a number of mammalian strains (411) have limited the use of agglutination reactions for their general identification. Recognizing these difficulties, Wilson (1265) and Griffith (471) independently, in 1925, concluded, through the use of antibody absorption techniques, that *M. tuberculosis* and *M. avium* could be serologically distinguished one from the other, but that *M. tuberculosis* and *M. bovis* could not.

Although heat-labile surface antigens (the K antigens of Lautrop [695]) have not been characterized for mammalian strains, their existence is indicated by the fact that inagglutinable strains (i) may stimulate antibodies reactive with agglutinable strains, (ii) may adsorb these same antibodies, and (iii) may be rendered agglutinable by heat at 65 to 100°C (411).

Serology of *M. avium*

Furth (411) exploited both agglutination and complement fixation tests in the antigenic analysis of strains of *M. avium* with reference to *M. tuberculosis* and *M. bovis*. He found that avian strains fell into three subtypes. He felt that the bacilli isolated by Kedrowsky (605) and Duval (328) were also avian strains. In 1935, Schaefer began a serological study of avian strains. The early part of this work and its confirmation by others were summarized in 1965 (1019). Stable suspensions of *M. avium* cells (antigen) are obtainable; the agglutination is clear-cut, and absorbed antisera against three distinct types (775), used in proper dilution, give no confusing cross-reactions between types. Some constitutively chromogenic strains and nonchromogenic strains have been found to be identical as to serological type. Further, some chromogenic strains have been found to be fully virulent for chickens, whereas others have lost this virulence. Thus, there is no correlation between the capacity to synthesize carotenoids and virulence (for additional evidence on this point see section on Carotenoids). Avian types I and II both have been the cause of human disease. Although both types infect birds and swine, type II seems to occur more frequently as a cause of infections in chickens, other birds, cattle, swine, and man (1022). Until recently, the differentiation of *M. avium* and *M. intracellulare* was based on a pathogenicity test in chickens; *M. avium* was said to be pathogenic; *M. intracellulare*, not (1295).

Schaefer extended his serological grouping of mycobacteria to strains of *M. kansasii* and to certain other strains falling into Runyon's groups II, III, and IV (1020). By examining the capacity to absorb agglutinins and to be agglutinated, certain cross-reactions among these mycobacteria were analyzed, and the number of serotypes was thereby extended (1021). The serological limits for *M. avium* at present appear to be set at three serotypes (1279). Fourteen of eighteen serotypes other than *M. avium* were encountered not only in man but also in cattle and swine. Those strains isolated from cervical lymph node infections in children (Table 4) fell into either of two serotypes. Thus, by 1967, it was evident that serotyping of these mycobacteria furnished essential adjunct information for clinical diagnosis of mycobacterial infections. In fact, Fischer et al. made this clear in their presentation of 50 of 1,185 consecutive admissions (to the Tuberculosis Service at National Jewish Hospital) who continually excreted mycobacteria other than *M. tuberculosis*, *M. bovis*, or *M. avium*. Of these, 29 were *M. kansasii* and the remaining organisms fell into 10 separate serotypes (376). Birn et al., in dealing with the *M. avium-M. intracellulare* complex, have expressed their frustration with serotyping in relation to the test for pathogenicity in the chicken by using such terms as "opportunist[ic]" mycobacteria, making them synonymous with nameless mycobacteria and suggesting "a broad division" of such bacteria to be defined as "dysgonic nonchromogens." They found that chromatographic patterns of lipids were of some help in distinguishing certain avian groups (134). Jenkins et al. have attempted to further delineate some of the rapidly growing mycobacteria by chromatographically examining their lipids in relation to their agglutination reactions (550). Although the lipid analyses of these authors amount to no more than resolution by TLC of spots with three solvents, (i) propanol-ammonia-water (75:3:22, vol/vol), (ii) butanol-acetic acid-water

(60:20:20, vol/vol), and (iii) propanol-isopropyl ether-water-ammonia (45:45:9.5:0.5, vol/vol), distinctive patterns have been obtained, and these correlate well with patterns of agglutination by absorbed specific sera. It would be a great advance to know the chemical nature of these lipids.

In a study of endemic tuberculosis in birds (1023), expected lipid patterns did not always occur in extracts from strains of a specific serotype. In some cases, a lipid pattern suggestive of *M. avium* was found, but the cultures were too rough to permit reading of agglutinations. This paper illustrates the importance of careful microbiology in the early stages of isolation of the mycobacteria, and the authors state that "much labour will be saved in the typing of *M. avium* strains by first plating on oleic acid-albumin agar medium. The selection of a *transparent* colony for the examination will give the best chance of successful typing by agglutination or lipid analysis" (1023). Actual characterization of the lipids responsible for the patterns found would advance considerably our understanding of the structure of the bacteria. Information regarding the serology of delipidated strains might help in the long-term objective of equating mycobacterial serotypes and chemotypes.

Schaefer et al. have shown the value of serotyping for analyzing 109 cases of *M. avium-M. intracellulare* infections (89 were genuine, of which 32 were *M. avium*) in England and Wales (1024). Reznikov et al. have confirmed the utility of the serotypes of Shaefer et al. and have employed them to relate strains of the *M. intracellulare-M. scrofulaceum* group isolated from house dust to those isolated from sputa (969). Reznikov and Leggo have suggested certain modifications in the procedure for serotyping of organisms of the *M. avium-M. intracellulare-M. scrofulaceum* complex (968), and Reznikov and Dawson have since found additional serotypes (967). The overall efforts of the investigators just discussed have produced a growing list of serologically distinguishable mycobacterial strains among the intergradations that constitute the *M. avium* complex (*M. avium*, *M. intracellulare*, and *M. scrofulaceum*). Wolinsky and Schaefer have recently suggested a scheme for simplifying the designations of the antigens of this complex so that each antigen would be represented by an arabic numeral: *M. avium*, 1, 2, 3; *M. intracellulare*, 4, 5 . . . 19, 20; *M. scrofulaceum*, 41, 42, 43 . . . (1279).

By dint of hard work, then, over a period of 14 years, the long-studied *M. avium* has offered us the first mycobacterial species readily identifiable by simple agglutination procedures. Unfortunately, there are some drawbacks. For example, the pathogenicity of *M. avium* for chickens is not correlated with serological type. Some strains of *M. avium* may be chromogenic. Both pathogenic (for the chicken) and nonpathogenic chromogenic strains have been found. Although type I and type II strains cause disease in man, cattle, swine, and birds, type II strains are the most infectious and pathogenic of avian tubercle bacilli (1020). The feasibility of serotyping of *M. avium* strains has led to a basis for a workable system of serological identification for *M. kansasii*, *M. intracellulare*, *M. scrofulaceum*, and related mycobacteria. Anz et al. have successfully carried out serological studies of avian strains isolated in West Germany by employing a number of reference antigens including *M. scrofulaceum*, *M. intracellulare*, *M. kansasii*, and *M. marinum*, as well as standard avian strains (49). Aho et al. have employed antigens prepared from nine strains of *M. intracellulare* for screening for circulating agglutinins in patients and healthy persons in Finland. Agglutinins occurred in 40 to 67% of the population and without any correlation with illness. The antibody spectrum varied from person to person, suggesting that a particular person's antibodies were the result of "immunization by the mycobacterial type(s) used for serological testing or a closely related type" (16). Schröder and Magnuson have demonstrated the usefulness of agglutination as the final step in distinguishing *M. kansasii* from other photoinducible chromogenic mycobacteria and for the identification of strains of *M. kansasii* blocked in the biosynthetic step from lycopene to β-carotene (see section on Carotenoids) as well as colorless mutants of that mycobacterial species (1033).

Schaefer et al. (1028) have shown the value of agglutination and absorption in assembling representatives of a species which they have designated *Mycobacterium szulgai*. They reported that antisera against *M. szulgai* agglutinated only *M. szulgai* and one other mycobacterium which, however, was unable to remove by adsorption to any degree the specific *M. szulgai* agglutinins. *M. szulgai* has one paradoxical property. It is constitutively chromogenic at 30°C but requires light for formation of pigment at 25°C.

Juhlin and Winblad have evolved a mycobacterial serotyping technique that is designed to conserve antiserum (consumes one-tenth that used in tube agglutinations) and avoids problems with unstable antigen suspensions and the need to deal with living mycobacteria. The

two interactants are (i) a strain of *S. aureus* killed and coated with serologically active *antimycobacterial antibody* and (ii) killed mycobacteria as the *mycobacterial antigen*. It has been known for sometime that the A-protein of *S. aureus* (produced by about 98.9% of coagulase-positive strains and by about 2% of coagulase-negative strains [389]) has the capacity to fix the Fc-end of about 45% of the IgG molecules (390, 1269), leaving the Fab portion free to react with antigen. In the method under discussion, specific spot agglutinations were effected using 3 drops of a mycobacterial suspension (heat killed) and 3 drops of the suspension of antibody-coated staphylococci (plus a saline control of mycobacterial antigen). The results were reported to be clear-cut and easy to read (569).

Soluble Antigens (Immunodiffusion)

In 1958, Parlett and Youmans (908) studied the antigenic relatedness of a number of mycobacterial species by using agar gel diffusion. They confirmed the independent findings of Wilson (1265) and Griffith (471) that *M. tuberculosis* and *M. bovis* are antigenically very closely related, and they made the astute observation that constitutively chromogenic strains, photoinducible chromogens, and nonchromogenic strains were associated with no single serological group. In the same year Castelnuovo et al. (222) combined immunodiffusion (ID) and immunoelectrophoresis for examining mycobacterial antigens. Since then, the need for characterizing the antigens being detected, i.e., knowing what they are, has been well appreciated but seldom realized. More recently, soluble antigens have been separated from mycobacterial cells, fractionated by a variety of procedures, and then employed in precipitin reactions, commonly in agar gels, or adsorbed onto particles and used in agglutination reactions. Yoneda and Fukui (1296) have reported and reviewed the isolation and purification of two protein antigens from $H_{37}Rv$, which they termed α and β. Although they found weak catalase activity associated with the β antigen, they ascribed enzyme activity to neither of these proteins. Employing anti-α and anti-β antisera, they have found homologous antigens in *M. bovis* strains Ravenel and BCG, but not in *M. kansasii*, *M. phlei*, *M. smegmatis*, or *M. fortuitum*. By now, a large literature has grown up from surveys for relatedness among mycobacteria by examining reactions between their antigens and homologous antibodies in agar gels. See, for example, 60,000 individual tests reported by Kwapinski et al. (680). Extensive immunodiffusion studies of *M. avium* in relation to *M. kansasii*, *M. simiae*, *M. marinum* (*balnei*), *M. smegmatis*, and *M. phlei* have been reported by Weiszfeiler et al. (1243; see also [583]). Bacteriophage lysates of mycobacteria have been recommended as a ready source of precipitins for analysis by ID (405). Turcotte has pointed out that the antigen profiles of bacterial extracts vary with time of harvest in relation to age of cultures and that, sometimes, known antigens fail to make their appearance in the culture filtrate or in extracts of ground cells (1180). Turcotte and Boulanger (1181) have used cross-absorption of antisera followed by immunoelectrophoresis as a means of examining the number of antigens detectable in $H_{34}Rv$, Ravenel, and *M. avium* (Sheard), and the avirulent homologues of each. Each of the virulent strains produced at least two antigens not found in the homologous avirulent strains. What are these antigens? As has been found off and on for the past 50 years, *M. tuberculosis* and *M. bovis* were antigenically close, whereas each was rather distinct (antigenically) from *M. avium*.

Lind et al. have found that incubation temperature quantitatively affects the antigenic composition of a variety of mycobacterial cells, as well as that of their associated (culture) filtrates (727). Norlin and Ernevad (884) have demonstrated the feasibility of separation of mycobacterial antigens by gel filtration and zone electrophoresis. First-run fractions were screened by ID. Sometimes more than one antigen came off in the same fraction. Subsequently, each of these was separated. Through various modifications of their procedures a number of "purified" antigens was obtained. For example, the following antigens were recovered in a *serologically* pure state: from *M. avium*, α, β, γ, and d; from *M. marinum* (*balnei*), α and b; and from *M. fortuitum*, e and β. Obviously, relatively homogeneous antigens and absorbed monospecific antisera are useful reference materials needed in carrying out these valuable adjunct methods for the identification of mycobacteria. In this work, only single strains of *M. avium*, *M. marinum* (*balnei*), and *M. fortuitum* were used. When more representative samples of *M. avium* (from both serotypes 1 and 2) were employed in a subsequent study concerning *only M. avium* in relation to *M. intracellulare* (serotypes 3 to 7), very different distributions of the key antigens (α, β, γ, d, and e) were found. In this work, Kubin et al. (673) employed the supernatants derived from cells broken for analysis by immunodiffusion. They found three strains of serotypes 1 and 2 which lacked antigen d, one which failed to

show any γ activity, and four strains which contained e antigen. These results were very different from their early reports based on single strains. The authors acknowledged that agglutination reactions served to better delineate *M. avium* and *M. intracellulare* strains than did their analysis of precipitinogens by ID. This is understandable since, in their work, most of the tightly bound antigens of the mycobacterial cell surface were eliminated during centrifugation of fractured cells to produce the soluble (intracellular) material used in their ID analyses.

Wayne (1227) has found that antigens, probably from the surface of *M. kansasii*, *M. gastri*, and *M. marinum* can be extracted in phenol. Such phenol-extractable antigens had the capacity to absorb agglutinins specific for the species under study, thus suggesting that phenol extraction might be a useful first step in the isolation and characterization of some mycobacterial antigens. Affronti and associates have brought the application of two-dimensional polyacrylamide gel electrophoresis (1286), two-dimensional immunoelectrophoresis (2D-IEP) (986), and discontinuous pore gradient gel electrophoresis (15) for establishing patterns of molecular migration characteristic of various mycobacteria. Using these methods, the authors have uncovered a wide variety of components categorized as protein, carbohydrate, lipid, and nucleic acid. The interaction of some of these components with antisera has been studied, and at least one "band" common to several mycobacteria was found. Whether or not this represented the common mycobacterial antigen found by Castelnuovo et al. (224) remains to be ascertained. The need for standard reference mycobacterial antigens has been felt and, towards this end, "reference culture filtrates" (RCF) and reference antisera have been produced under the aegis of the United States-Japan Tuberculosis Panel (545). Daniel and Affronti (278) have made a comparative immunoelectrophoretic analysis of the U.S.-Japan RCF with fractions of $H_{37}Ra$ prepared by the method earlier used by Seibert (1043). By reaction identity, Seibert's five fractions could be related to RCF antigens as follows: Seibert protein A = RCF 1, 2, 5, 6; protein B = RCF 1, 2, 5, 6, 7; protein C = 2, 6, 7; Seibert polysaccharide I = RCF 1, 2; polysaccharide II = RCF 3. Years earlier, Birnbaum and Affronti (135, 136) had shown that tuberculopolysaccharide I of Seibert was undoubtedly arabinogalactan from the cell wall (see section on Cell Wall) and that there was a common identity by ID between the arabinogalactans of $H_{37}Ra$, *M. kansasii*, and *M. intracellulare* (see also legend to Fig. 20).

The technique of 2D-IEP, in which a complex of antigens is subjected to electrophoresis through an agarose matrix in one direction and then through an antiserum-agarose matrix at right angles to the first direction, was successfully applied by Roberts et al. in a comparative study of antigens and antigen-antibody precipitates of *M. tuberculosis*, *M. bovis* BCG, *M. scrofulaceum*, and *M. phlei*. The superiority of 2D-IEP was evidenced by the fact that it revealed 36 precipitin peaks for *M. tuberculosis*, whereas one-dimensional IEP yielded only 11 to 13 immunoprecipitates (986). Wright and Roberts (1287) have found that it is possible by 2D-IEP to employ one standard reference serum (prepared in goats against $H_{37}Rv$) for use in comparative studies of extracts (prepared by sonic treatment of cells) from several different species of mycobacteria.

Ridell and Norlin have shown the usefulness of mycobacterial reference precipitinogens for examining serological relatedness between *Mycobacterium* and *Nocardia*. They have given their own α, β, γ, and δ designations for antigens demonstrated by immunodiffusion. They have found that 24 of 56 nocardias possessed the mycobacterial antigen α, a different 30 of 56 produced mycobacterial antigen partial α (pα) and 29 of 56 produced mycobacterial antigen β. Significantly, only certain strains of *N. farcinica* (5 of 8) produced β antigen, and these strains the authors considered for several reasons to be mycobacteria (981; see also [228]).

Stanford and various associates have employed immunodiffusion methods for the "antigenic analysis" of strains of *M. fortuitum*, *M. kansasii*, *M. phlei*, *M. smegmatis*, and *M. tuberculosis* (1083); they have used similar methods, in conjunction with fermentation reactions and other bacteriological procedures, for studies of *M. friedmannii* (see Table 2 [1084]), *M. acapulcense* (see Table 2), *M. flavescens*, and certain "new" species (1086), *M. chelonei* (1087), *M. gordonae*, *M. scrofulaceum* (*marianum*), *M. avium* (479), and *M. fortuitum* (*ranae*) (1085). In many of these studies and in papers devoted to *M. ulcerans* (1081) and to *M. lepraemurium* (1082), a number of different strains of each taxon have been examined, thus greatly enhancing the value of the results.

It is very difficult in the search of immunological analyses of mycobacteria to find a study of antigens associated with an enzymic activity or a particular molecular structure such as a terminal *O*-methylfucose on a surface peptidolipid (see section on Peptidolipids). Yet, as early as 1964, Cann and Willox showed some promise for the exploitation of esterases separated by starch gel electrophoresis in the characteriza-

tion of four "mycobacteria" readily separable by other means (215). Nakayama later extended this approach to other mycobacteria including *M. tuberculosis*, *M. bovis*, *M. kansasii*, *M. avium*, and others. It is interesting that of the 8 species (71 strains in all), the zymogram for each species was distinctive, except for those of *M. tuberculosis* and *M. bovis*, which were alike (868). Surely, that is the kind of information we must have if we are to reach an understanding of the interrelationships that exist among mycobacteria. Thompson et al. have made an effort in this direction with the β-lactamase of $H_{37}Rv$ (1153) which, unfortunately, is an enzyme produced in low yields. Andrejew and Renard have shown that one can separate the catalase activity from the peroxidase activity of *M. avium* (46). Bailey et al. have found a strain of *M. kansasii* that is a hyperproducer of catalase (82). Bartholomew (99) has shown that *M. smegmatis* synthesizes what are probably two molecular species of catalase, but only one of these is found in *M. gastri*. If one mycobacterial catalase were prepared in crystalline form, it and its antiserum could be tested for identity with one of the many mycobacterial precipitinogen antiprecipitinogen lines known cryptically as α, β, γ, etc., as well as tested for examining the relatedness of isolated catalases. (Wayne and Diaz [1230] have recently produced immune sera against the catalase of $H_{37}Rv$. The antibody precipitated but did not inactivate the enzyme. Preliminary studies with catalases from other mycobacteria indicated differences in capacity to bind to the antibody. Some species produced catalase that did not react with the $H_{37}Rv$ antiserum. No effort was made to relate the catalase of *M. tuberculosis* to such established antigens as α, β, γ, etc.) Other candidates for use in bringing meaning to the accumulated antigens discovered by ID could be the lysine decarboxylase of *M. tuberculosis* (1261), the Ca^{2+}-activable esterases of *M. phlei* (943), the DNA polymerase of *M. smegmatis* (807), the lactate oxygenase of *M. phlei*, etc. The versatile lactate oxygenase of *M. phlei*, which catalyzes the oxygenative conversion of L-lactate to acetate and, under anaerobic conditions acts as a lactate dehydrogenase (1127), was first crystallized in 1957 (1114). This flavoprotein, molecular weight 350,000, is a hexamer of six equivalent subunits (molecular weight 54,000 to 57,000) each having one N-terminal serine and one C-terminal arginine (1129), of which Katagiri et al. (587) have obtained elegant electron micrographs. Katagiri et al. have previously described methods for obtaining the enzyme in good yields (1128). Thus, mycobacterial lactate oxygenase is a candidate for comparative studies between species (e.g., do other mycobacteria such as *M. smegmatis* [lactate oxygenase crystallized in 1968, as in reference 1110], BCG, *M. kansasii*, and *M. avium* produce identical or slightly modified lactate oxygenases? Do these account for some of antigen-antibody lines picked up in the various ID surveys already referred to?)

Although ID lines of common identity can mean just that, a more useful meaning comes from knowing what kind of biological activity characterizes any antigen that produces a precipitate with its homologous antibody. Furthermore, comparative ID studies should employ known amounts of antigen and known amounts of antibody. Otherwise, an absence of antigen-antibody precipitate in testing for cross-relationships may be merely a matter of inadequate concentration of one or the other immunoreactant. Concerning such matters, Stanford and Gunthorpe have published a classroom example. They have described a new species of *Mycobacterium* principally on the basis of ID lines: "The species is characterized by the possession of 6 species-specific antigens demonstrable by immunodiffusion tests." The *specificity* of these anonymous antigens was documented by four immunodiffusion plates on which were ID lines generated by lysates (and "homologous" antibodies) from five "species" of mycobacteria (1086). For a picture of this mycobacterium, see Fig. 16A.

Agglutination of Particles Coated with Soluble Mycobacterial Antigens

Middlebrook and Dubos (818) initiated the use of erythrocytes coated with mycobacterial antigens (derived from $H_{37}Rv$) for agglutination reactions (hemagglutination) aimed at gaining more information about the immune response in tuberculosis. Suitable titers of antibody to these antigens were found in patients and in experimental animals. There seems to be an inherent desire among investigators to find a single test that will give the answer. When such is not found, another search is started. A paper written by Froman and coworkers some years ago points up the complexities of serological testing in tuberculosis (404). They employed (i) formalinized erythrocytes (human group O, Rh-negative) coated with material adsorbed from old tuberculin (OT), (ii) kaolin particles associated with phosphatides from *M. tuberculosis* (phosphatide-kaolin agglutination test), (iii) Sauton culture filtrates and extracts from sonically treated cells (for double diffusion in agar) of *M. tuberculosis* $H_{37}Ra$. The subjects tested were: *group A*, 138 bacteriologically positive patients diagnosed as

tuberculous; *group B*, 94 patients diagnosed as tuberculous from whom *M. tuberculosis* could not be isolated; and *group C*, a control group of 47 tuberculin-negative adults. Positive reactions to one or more of the tests occurred in 85% of group A, 75% of group B, and 6% of group C. More than 50% of group A patients were positive for two or more of the tests, and an additional 30% were positive to only one of the three tests; of the group B patients, 38% were positive to two or more tests, and an additional 38% were positive for one of the three tests. In the control group, those positive (6%) were positive to one only of the tests. Although the data do not indicate that *the* test has been found, they do point up an interesting difference among the bacteriologically positive patients. One would like to know more about the 15% nonreactors. The authors raised the question as to whether or not this hard core of nonreactors (i) might have responded to other tests not employed by them, (ii) might have contained antigen-antibody complexes, or (iii) might have had blocking levels of circulating incomplete (blocking) antibody. The possibility of an immunological unresponsiveness shared by this 15% was not suggested as the basis for their failure to make detectable amounts of antibody.

The results of Ishibashi et al. (538) employing the hemagglutination reaction between wax D adsorbed on erythrocytes and antisera directed against *M. tuberculosis* Aoyama B indicated that the (lipo) "polysaccharide antigen" of Middlebrook and Dubos (818) shared determinants with those of wax D.

Daniel (277) used hemagglutination as a means of measuring the immune response of New Zealand white rabbits to OT, OT in incomplete adjuvant, alum-precipitated PPD, and killed $H_{37}Rv$. A measurable primary response occurred in each of the animals. The authors found only IgM formed in the animals immunized with soluble OT and alum-precipitated PPD. OT in adjuvant and killed $H_{37}Rv$ elicited the formation of both IgM and IgG. Ten weeks postimmunization, all IgM had disappeared, and there were residual levels of IgG in those animals in which it had been formed. When the animals were reinoculated at 13 weeks, none showed a secondary response, probably because of still-persisting antibody available for the formation of immunosuppressive immune complexes.

The Soluble-Antigen Fluorescent-Antibody Test

The soluble-antigen fluorescent-antibody (SAFA) test for circulating antimycobacterial antibodies employs cellulose acetate filter disks impregnated with proteins A and C (14), as well as a partially characterized arabinogalactan (from cell walls, see Birnbaum and Affronti [135, 136]), all from $H_{37}Ra$ (373; see also [1159]). The moist, impregnated disks are reacted with dilutions of sera to be tested. Following storage in the cold, disks are washed, rendered relatively dry, and then exposed to fluorescein-labeled antiglobulin antibody (anti-human, anti-monkey, etc., according to subject animal). Final reading for level of antibody in the immunological sandwich (antigen-antibody-fluorescing anti-antibody) is carried out in a fluorometer. Affronti et al. (13), using a modified SAFA test, have shown that 11 of 12 monkeys having fatal tuberculous infections developed a detectable antibody response, and 10 of these responses were detected by day 42 postinfection. The SAFA test was positive earlier than the tuberculin skin tests (both intradermal palpebral and intradermal abdominal injections were carried out on each animal). The SAFA test was also positive for 25 tuberculous patients hospitalized for chemotherapeutic treatment. Furthermore, these patients showed a significant increase in SAFA-detectable antibodies within 30 to 90 days posthospitalization.

Tuberculins and Other Mycobacterial Elicitins

Skin test reactions. Magnusson (756) has suggested the term "sensitin" for "a nonantigenic substance, prepared from a microorganism (virus, bacterium, or fungus), capable of revealing sensitivity of the delayed type evoked by the organism." Since these products elicit skin reactions but do not sensitize for such reactions, "elicitin" would seem a more apt term than "sensitin." Human and avian tuberculin, histoplasmin, and coccidiodin are examples of elicitins. The model of this kind of immunological detection is found in the guinea pig experimentally infected with *M. tuberculosis* and skin tested with PPD. Since the number (kinds) of sensitizations induced by the infecting organism is usually multiple, the elicitation of specific response in the skin of that animal is dependent on the purity of the eliciting reagent, lack of cross-reactions, etc. (see Table 6).

By 1957, at the State Serum Institute in Copenhagen, the following protocol was in use for the analysis of interrelationships among mycobacteria based on the degrees of specificity in skin tests. Sensitization was induced by the intradermal injection of dried, heat-killed mycobacteria suspended in paraffin oil. Follow-up skin tests were carried out 3 to 4 weeks later

TABLE 6. *Elicitins (PPDs) prepared from various strains of mycobacteria and used for skin testing recruits in the U.S. Navy*[a]

Mycobacterium (elicitin source)	Elicitin designation	No. tested	Reactions of 2 mm or more	
			%	Mean size (mm)
M. avium	PPD-A	10,769	30.5	6.7
M. fortuitum	PPD-F	3,415	7.7	4.8
M. intracellulare	PPD-B	212,462	35.1	7.7
M. kansasii	PPD-Y	13,913	13.1	6.2
M. phlei	PPD-ph	15,229	23.1	6.4
M. scrofulaceum	PPD-G	29,540	48.7	10.3
M. smegmatis	PPD-sm	14,239	18.3	5.7
M. tuberculosis	PPD-S	212,462	8.6	10.3

[a] Adapted from Edwards (333): the standard dose was 0.0001 mg of the indicated PPD. Almost half of the persons tested gave a response to PPD-G. The cross-sensitivities indicated in these data complicate the interpretation of tuberculin reactions. See also section on Mycobacterial Sensitins.

using dilutions of PPD-like preparations derived from such mycobacteria as *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. xenopi*, *M. flavescens*, *M. fortuitum*, *M. smegmatis*, and *M. phlei*. Cross-reactions were resolved by calculating the difference between the sums (average diameters in millimeters) of the homologous and heterologous reactions (756, 757, 759). Burulin, a skin test reagent useful in detecting specific DH in patients infected with *M. ulcerans*, has been described by Stanford et al. (1088).

PPD-S, OT, and their tuberculin-active components. Thirty years of experience with PPD-S (= PPD from *M. tuberculosis*) has shown the "product" to be compound, and commercial preparations of it have been found to vary in the multiplicity of their components (432). Ever since Baer and Chaparas found that dialyzable low-molecular-weight components from BCG filtrates had the capacity to elicit a tuberculin reaction (78), the difficulties in obtaining a pure monospecific eliciting agent have seemed great. At present, the specificity of PPD-S is not such that a positive skin test response to it (in human populations) necessarily indicates an immunogenic experience, past or present, with *M. tuberculosis* (34, 334, 903) (see Table 6). Moulton et al. have prepared PPD according to the method of Seibert and Glenn (1044) and further separated it by Sephadex G-200 into three fractions. The capacity of each fraction to elicit delayed hypersensitivity was tested in guinea pigs sensitized with *M. tuberculosis* and in guinea pigs sensitized with *M. kansasii*. Fraction I elicited almost equally reactions in the *M. tuberculosis*-sensitized and in the *M. kansasii*-sensitized guinea pigs. This fraction contained two immunologically reactive components. Fraction II gave a mean homologous reaction of 12.1 mm and a cross-reaction of 4.4 mm and contained two cathodic-moving components. Fraction III, a single anodic-moving component, gave a mean homologous skin reaction of 9.8 mm and a cross-reaction of 1.8 mm. The greatest immediate (Arthus) reaction elicited (6 h) by either of the fractions was 5.2 mm. Pronase digestion eliminated the capacity of each of the three components to elicit positive skin tests (860). To date, the most defined component(s) found to make possible sensitization by PPD-S to PPD-S is polyadenylate:polyuridylate (218).

Magnusson (758) has used the delayed skin reactions in sensitized guinea pigs for examining the elicitins of *M. gastri* and certain strains of *M. kansasii*. *M. gastri* appeared separable from *M. kansasii*. *M. kansasii* and certain of its white mutants appeared to make identical elicitins. This suggests that a mutation in the carotenoid biosynthetic pathway is without a detectable effect on the production of DH-eliciting peptides, glycopeptides, peptidoglycolipids, or whatever class of compounds comprise the elicitins of *M. kansasii*. Magnusson and Mariat (760) employed elicitin tests (DH) for discriminating *N. farcinica* from other nocardias. The tests indicated that a number of strains received by them as *N. farcinica* were closely related. What they failed to do was to include other representatives of the CMN group in their testing (see [981] under ID above), thus missing the opportunity to discover mycobacterial immunoreactive components in *N. farcinica*, which is now accepted as *M. farcinogenes* (228).

Elsewhere, we have pointed out that culture filtrates of *M. tuberculosis* $H_{37}Rv$ yield 36 immunoprecipitin peaks on 2D-IEP. Which of these are tuberculin active? The answer is not yet available. However, Chase and Kawata (240)

have begun a rational approach to the problem of sorting tuberculin-active mycobacterial antigens. They used a set of IgG_1 antimycobacterial sera for ascertaining the antigenic complexity of "tuberculins" (tuberculin-active preparations) through a carefully quantified technique of passive cutaneous anaphylaxis (PCA) carried out in guinea pigs. Animals lacking immune experience with mycobacterial antigens are intracutaneously prepared at various sites with different batches of IgG_1 immune sera. (Sera corresponding to at least 10 antigen-antibody systems were used. Reactivities of different sera were adjusted to equivalent concentrations. A range of concentrations was employed to accommodate to variations in dermal behavior from animal to animal.) The prepared animals received, intravenously, antigens under investigation. The firing, or not, of PCA reactions at different sites provided comparative data for an immunological analysis of the various PPD-Ss and OTs being tested. An examination of batches of PPD-S (prepared in several ways: e.g., by precipitation with ammonium sulfate, trichloroacetic acid, or benzoic acid) from several sources indicated the presence of at least five different antigens (certain unheated preparations contained more). OT could be shown to contain one dominant antigen not present in all PPD-S. OT seemed to lack one dominant antigen found in all PPD-S. The dominant antigen of OT was not found in trichloroacetic acid-PPDs but was present to some degree in most PPD-Ss prepared by ammonium sulfate precipitation. The authors conclude that infection with *M. tuberculosis* leads to the acquisition of several delayed-type hypersensitivities. They suggest that OTs and certain PPDs test for different DHs and that at least five types of tubercle bacilli-induced DH exist. It is interesting to note that OT detects a particular DH sensitivity. A number of years ago, one of us (L. B. in collaboration with E. L. Hopphan and T. Rei) found that a much-earlier-signal DH for sacrificing guinea pigs that were developing tuberculosis from clinical materials could be obtained by testing with *OT* than with various strengths of *PPD*. It would be interesting to know now whether the distinctive tuberculin-active component found in OT by Chase and Kawata can be related to a sequence of DHs (early, middle, late) that develop following tuberculous infection in the guinea pig and other sensitive animals.

The need for highly purified tuberculin-active peptide has long been felt. Morisawa et al. reported in 1960 (853) the crystallization from culture filtrates of $H_{37}Rv$ (grown in Sauton medium) a heat-stable, basic peptide capable of eliciting DH in human subjects in amounts of 0.1 μg. More recently, Kuwabara has reported the purification and properties of a tuberculin-active protein, molecular weight 9,700, obtained in crystalline form from acetone-powder preparations of heated cells of *M. tuberculosis* Aoyama B. Cell-free extracts were exposed to DNase and RNase following treatment with streptomycin. Redissolved precipitate from 75% saturated ammonium sulfate was passed through diethylaminoethyl (DEAE)-cellulose columns and then through Sephadex G-200. Crystallization was from eluates of the latter. The final product, a crystalline protein, contained an estimated specific activity of 6.33×10^9 tuberculin units per mg of protein nitrogen for guinea pigs and 6.33×10^{11} tuberculin units per mg of protein nitrogen for sensitized human subjects (678). Subsequently, the amino acid sequence was determined. Among the seven tryptic peptides found, only one, consisting of Asn-Gly-Ser-Gln-Met-Arg, exhibited tuberculin activity. No information was given regarding the immunogenic capacity of the protein (679).

Although the growing need for chemically pure and well-characterized eliciting substances for exploiting the exquisite specificity possible in tuberculin-type reactions has been felt by many investigators, model systems worthy of the term "reference systems" are still scarce. One such model could derive from recent studies of *M. avium* and *M. paratuberculosis*. It has been on record since the report of Hagan and Zeissig (481) and the opinion of Paterson (909) that some animals infected with *M. paratuberculosis* (*johnei*) respond to elicitins from *M. avium*. In fact, the reactions in the skin caused by these two preparations have been said to be indistinguishable (909). Singleton et al. (1057) have examined the capacities of peptidoglycolipid fractions from *M. avium* and from *M. paratuberculosis* to elicit DH reactions in guinea pigs sensitized to *M. avium* and to *M. paratuberculosis*. By this test, the active principal in the two fractions seemed identical. This similarity of behavior was paralleled by an overall similarity in the infrared spectra of the two. Although the authors termed the materials with which they worked as peptidoglycolipid, implying a mycosidic nature for the compounds involved, the material actually consisted of murein and polysaccharide from the mycobacterial cell wall (reported [991a] as glutamic acid, alanine, *meso*-diaminopimelic acid, glucosamine, galactose, mannose, arabinose, aspartic acid, and traces of glycine and phenylalanine) and considerable lipid. Consequently, their results only mean that their fractions

from *M. paratuberculosis* and *M. avium* had much in common. They were not dealing with the specific, superficial mycosides or peptidoglycolipids. Lanéelle (687a, 689a) has demonstrated that *M. paratuberculosis* produces peptidolipid but not peptidoglycolipid (see section on Glycolipids, Peptidoglycolipids, etc.).

Kubica et al. (668) have shown that mice sensitized by the injection of viable mycobacteria into one of the hind footpads responded (3 or 4 weeks later) to a second injection in the contralateral footpad with an accelerated response that was exaggerated over that of the first injection. Tuberculin shock following testing is one of the hazards of the procedure. The authors suggest that this can be obviated by using 10 mice per test and making the sensitizing and challenging doses around 1,000,000 viable units per mouse.

THE IMMUNE RESPONSE TO *MYCOBACTERIUM*

"Humoral Immunity" (HI), the Plasma Cell Arm of the Immune System

The immune response to *chronic* infections begins with inflammation and involves the entire gamut of the defenses of the host. The effectiveness of these defenses varies according to the genetic makeup of the host animal (740) and that of the invading agent. The regulation of the mechanisms of defense may be affected in various ways according to the offending agent. In rabbits, guinea pigs, and other susceptible animals, experimental tuberculous infection, like so many other bacterial infections, begins with a pouring in of polymorphonuclear leukocytes (PMNs) at the site of injection (305) and with subsequent ingestion of the bacilli by these cells. The bacteria-laden granulocytes subsequently die, and their remnants and contents are engorged by macrophages (973). There is little or no evidence to suggest that, before dying, these PMNs have had a lethal effect on their engulfed mycobacteria (973, 488), despite the hydrogen peroxide such PMNs apparently liberate (616).

During tuberculous infection, antibodies are formed (818), and immunoglobulins reactive with *M. tuberculosis* are reported to be common among "normal" human subjects (93, 393). The importance of antibodies in recovery from tuberculosis has long been questioned. Some years ago, Raffel reported experiments which suggested that infected guinea pigs passively treated with repeated doses of antituberculous sera (as well as whole blood) developed a tuberculous infection comparable to that in control animals receiving normal serum, normal whole blood, or nothing (954). Recently, Reggiardo and Middlebrook have reported that immune rabbit sera passively administered 1 day prior to, and on days 5, 11, 18, and 25 following, aerogenic challenge with *M. bovis* Ravenel failed to modify the number of viable bacilli recoverable from the animals at 6 weeks (964). These authors have been careful to point out that their results do not accord with: (i) those of Rowley et al. (993), who found in mouse typhoid (*Salmonella typhimurium*) that the specific aspect of immunity was related to a cytophylic antibody capable of fixing to macrophages and affording passive protection; (ii) those of Lurie (738, 739), confirmed by Tsuji et al. (1161), demonstrating in immune sera of guinea pigs and rabbits a humoral antibacterial component; and (iii) those of Fong et al. (386), showing that a factor in sera from *Salmonella*-immune rabbits in vitro nonspecifically protected macrophages from the necrotizing action of virulent *M. tuberculosis* $H_{37}Rv$. The additional finding of the latter investigators that "monocytes" from animals immunized with BCG and cultivated in anti-BCG serum were able to cope with $H_{37}Rv$, whereas "monocytes" from *Salmonella*-immune rabbits under the same conditions failed to withstand $H_{37}Rv$ infection, pointed to a role for both serum factors and mononuclear cells in the immune response. This archetypal finding, after being many times rediscovered, now seems to be generally accepted.

Antibody Responses in Tuberculous Infection

The mammalian immune system and its controls are complex and include mechanisms yet to be understood. These controls appear to involve complicated interactions between two classes derived from lymphoid stem cells: T, which have had an association with the thymus or thymosin (437, 633), and B, which have had an extrathymic maturation (335). The B lymphocyte is characterized by at least three surface receptors: (i) receptors that bind the Fc region of Ig molecules and provide a site for antibody molecules, antigen-antibody complexes or aggregated Ig (102, 103, 104, 300), (ii) surface Ig receptors for specific antigens (886), and (iii) receptors for the C3 component of complement (131, 888). These bone marrow-derived lymphocytes can be stimulated to produce the lymphokine, monocyte chemotactic factor, (i) by mitogens carrying multiple binding sites, e.g., lipopolysaccharide or polymerized flagellin (see [369]), (ii) by antigen-antibody complexes or aggregated gamma globulin interacting with the Fc receptor, and (iii) by interaction of anti-

gen-antibody-complement complexes at the C3 receptor. The bridging or cross-linking of receptors is thought by some to be the key to stimulation, and such stimulation is affected by *nonmitogenic* as well as mitogenic molecules as long as they can cross-link the receptors (1212). (For other theories as to the basis for B cell activation, see [266a].) A B lymphocyte with an immunoglobulin having an affinity for a specific antigen, upon binding that antigen, may be stimulated to divide, giving rise to a clone of plasma cells (monoclonal stimulation) capable of synthesizing immunoglobulin of specific *antibody* activity(ies). B cells may also be generally triggered to mitosis by certain species of molecules operationally defined as mitogens (e.g., certain plant lectins [polyclonal stimulation] [265, 266]), as well as by C'3 (323) and lipopolysaccharides (846). Compounds capable of polyclonal stimulation have also been termed PBA or polyclonal B cell activators (266a), and many of them have long been known as adjuvants. Classes of adjuvants that have been termed "B cell oriented" (319) include *Bordetella pertusis*, *Propionibacterium parvum*, and *E. coli* lipopolysaccharide (24). IgM produced in mice in response to lipopolysaccharide is of broad affinity and low avidity (319a). Although the B lymphocyte prior to antigenic stimulation possesses Fc and C'3 receptors, the cells of clones (plasma cells) derived from that B lymphocyte have lost or have covered up the C'3 receptors (104, 845, 1050). Certain, if not all, of the IgG's synthesized by plasma cells are cytophilic; i.e., they fix by their Fc end to macrophages (see following discussion of Cytophilic Antibodies). Thus, a major component of HI or B cell(s)-related immunity can interact with macrophages.

Adjuvants classed as "T cell oriented" and including beryllium, CFA, lentinan, polyadenylic acid·polyuridylic acid [poly(A)·poly(U)], and retinol are effective in the stimulation of antibody only in animals unimpaired as to complement of thymus-associated T lymphocytes. For example, Cone and Wilson (258) found a 10-fold amplification of T rosette-forming cells in mice receiving antigen (sheep erythrocytes [SRBC]) plus poly(A)·poly(U). T lymphocytes, probably with macrophages, are the major biosynthetic machinery for DH and are also essential for maximal B cell response to certain antigens. Through experiments employing T and B cells in separate chambers linked by Nucleopore membranes, Feldmann (368a) has demonstrated that T and B cell cooperation can occur through soluble factors. Thus there appears to be no requirement for cell-to-cell contact. Further, he has suggested that complexes of IgM from T cells with antigen become bound to macrophages, and it is with this macrophage-bound complex that B cells interact and become stimulated to produce antibody. Finding this interaction between the T and B components of the immune system has led to the suggestion that T cells function as regulators: (i) sometimes as suppressors of an immune response (428, 429, 507, 508, 895, 1117) and (ii) sometimes as enhancers of an immune response (86). In addition, there is the killer T cell (KTC). The study of lentinan's (a fungal polysaccharide, a linear β-(1-3)-glucan polymer) antitumor activity has revealed that this T cell adjuvant acts on the helper function, causing T cell enhancement of B cell antibody synthesis, but does not increase the sensitization of KTCs in an allogeneic system (295, see also [368b]). Silver and Benacerraf (1054) have shown a comparable dissociation of T cell helper function and DH using deaggregated protein carrier and its hapten. Using the former as a tolerogen in low doses, they could show the inhibition of helper function in the face of retained DH. Thus, the regulation of antibody synthesis extends beyond the B cell function per se. (Recently, Munro and Taussig have offered support for the thesis that, in mice, antibody production is controlled by two functionally distinct cistrons in the I region of the major histocompatibility complex. One controls [i] T cell recognition of antigen and [ii] T cell mediators of cell cooperation; the other controls the B cell response to the T cell mediators [864]. Further, the controls that lead to tolerance, e.g., tolerance induced by antigen-antibody complexes, may be T cell mediated [Taylor and Basten in reference 318].)

"Cytophilic" and other antibody activities associated with tuberculous infection. Over a decade ago Boyden and Sorkin described an antibody that was cytophilic for the spleen cells of rabbits immunized with human serum albumin (HSA; 170, 171, 1076). Guinea pig cytophilic antibody produced in animals immunized with SRBC mixed with CFA was found to selectively bind to homologous macrophages, rendering them capable of adsorbing SRBC (169). Berken and Benacerraf showed that this cytophilic activity for macrophages was a property of the complement-binding 7*S*, γ_2-class of guinea pig immunoglobulin (IgG). The antibody-binding site for the macrophages was located on the Fc fragment and thus is a part of the H chains. Cytophilic activity is not complement dependent. Cytophilic activity was shown to be a property of opsonizing antibody, which allows the fixation of antibody to the cell mem-

brane in an orientation optimal for phagocytosis. Cytophilic antibodies were found in immune sera from rabbits and mice vaccinated with SRBC. Sensitization of macrophages was maximal with homologous antibodies, but cross-sensitization between species did occur (127).

Amos et al. (35) found that the sera of guinea pigs, sensitized to tuberculin (PPD) using PPD in Freund adjuvant (or better, BCG followed by PPD in Freund adjuvant) as well as to β-lactoglobulin, contained cytophilic antibodies capable of fixing to macrophages and rendering them sensitive to migration inhibition by either PPD or β-lactoglobulin. This migration inhibition could, of course, be passively transferred with serum and was not to be confused with the lymphokines (290, 324) and other nonantibody mediators of cellular immunity generated after lymphocyte activation. Heise et al. (502), using BCG-sensitized guinea pigs and PPD, showed that cytophilic antibody could render macrophages sensitive to inhibition by PPD. Mixtures of PPD and immune serum were inhibitory to macrophage migration but not cytotoxic for mouse L cells. Cytophilic antibodies tend to come off macrophages at 37°C (127), and this has raised some question as to their importance in vivo (335).

Binding of guinea pig cytophilic antibody (γ_2-immunoglobulin) to mycobacterial glycopeptide. Stewart-Tull et al. (using the same method Seibert employed for the isolation of "polysaccharide" I of *M. tuberculosis* [1043]), have isolated from culture filtrates of *M. tuberculosis* a glycopeptide (containing arabinose, galactose, glucosamine, muramic acid, alanine, glutamic acid, α,ε-diaminopimelic acid, and small amounts of aspartic acid and glycine). They have shown that this glycopeptide ("polysaccharide" I of Seibert) has an immunological closeness (near identity) with the peptidoglycolipid wax D (see section, From the Cytoplasmic Membrane to the Peptidolipid). They noted (1102) an affinity of the glycopeptide for guinea pig immunoglobulin (formed in animals receiving CFA and egg albumin) and subsequently showed (1101) that it is capable of binding to both the Fc and Fab fragments of γ_2-globulin (γ_2G). The ratio of Fc-binding to Fab-binding was 4:2. Since γ_2G from guinea pigs immunized with bentonite-ovalbumin complexes as well as that from guinea pigs receiving repeated injections of incomplete adjuvant also fixed to glycopeptide, the authors concluded that specific immunization with mycobacteria (in adjuvant) was not related to the effect. They examined other glycopeptide fractions for their γ_2-binding capacity. It appeared that, for satisfactory binding, muramic acid and arabinose were essential components of the glycopeptide. Recently, Davies and Stewart-Tull have shown that, when SRBC become coated with glycopeptide, they have an affinity for guinea pig γ_2G. In fact, SRBC-glycopeptide-γ_2G complexes were agglutinable by rabbit-antiguinea pig globulin, thus establishing a dual affinity of the glycopeptide for cell membranes and for γ_2G (291). Human sera from active cases of tuberculosis (from 2 months to 7 years duration) showed no affinity for mycobacterial glycopeptide (1101). The authors have not indicated whether or not the glycopeptide can bind to the surfaces of B or T lymphocytes or macrophages. The glycopeptide shares with a number of mediators of immune responses the property of being bifunctional. Waksman and Namba (1213a) have pointed out that the concept inherent in the term "amboceptor" is bifunctionality, a property common to opsonic IgG, to cytophilic IgG in its binding by its Fc end to the macrophage and by its Fab end to antigen, and to IgE in its binding to mast cells or basophils. Are the bifunctional molecules of mycobacterial origin capable of effecting similar binding in the animal host? Would the presence of such molecules exert a regulatory effect on an immune response?

In 1963, Benacerraf et al. reported that guinea pigs immunized *intraperitoneally* with egg albumin or hapten conjugates of protein antigens responded with the synthesis of IgG_1 antibodies (120). However, when the same antigens were administered with CFA, both IgG_1 and IgG_2 antibodies were produced. IgG_2 antibodies could fix complement, whereas IgG_1 antibodies could not (144). More recently, Stewart-Tull et al. (1097) have electrofocused globulins from normal and CFA-plus-ovalbumin-stimulated guinea pigs and determined the ratio of IgG_2:IgG_1 by polyacrylamide disc gel electrophoresis and densitometry combined with $E_{280\,nm}$ spectroscopy. They concluded that, indeed, significant differences exist between IgG_1 and IgG_2 populations in anti-ovalbumin serum of animals stimulated by CFA and those of nonimmune, normal guinea pigs. It seems inescapable that the difference must be attributed to CFA. Wilkinson, giving due credit to Stewart-Tull and White, in 1966 observed that the development of granulomas in guinea pigs following injection of antigen-adjuvant mixtures (CFA or bentonite) was correlated with a rise in IgG_2 antibodies (1260). Kotani and associates have referred to this latter as evidence that a statistically significant relationship exists between the production of IgG_2 and the

development of DH (654). They have established a similar correlation between the development of DH and IgG_2 in animals immunized with water-in-oil emulsions consisting of ovalbumin as antigen and cell walls or their water-soluble fractions from a variety of gram-positive bacteria. They and Ellouz et al. (342) have independently shown that an ultimate adjuvant for inducing this effect is *N*-acetylmuramyl-L-alanyl-D-isoglutamine (655). Is there any relationship here between the affinity of IgG_2 for peptidoglycolipid-containing *N*-acetylmuramic acid and the increase in IgG_2 antibodies?

Cytotoxic antibody. Recently it has been found that humoral antibody (IgG) can induce and/or inhibit an in vitro lymphocyte-mediated cytotoxicity by thymus-independent cells (reviewed by Perlmann et al. [920; see also reference 1060]). In this lymphocyte-mediated killing, normal lymphoid cells are cytotoxic for particular target cells in the presence of antibody to the latter. The Fc portion of the antibody molecule is essential for cytotoxicity. Lymphocytes in contact with antibody-treated target cells showed increased mobility, as indicated by their infiltration of the monolayers of the latter. The activation of these infiltrating lymphocytes may come from interaction with antigen-antibody complexes on the surface of the target cells. Antigen-antibody complexes are known to stimulate lymphocytes to increase DNA synthesis (145, 844). Although the exact nature of the effector or killing lymphocyte is not known, it seems not to be a T cell (1060). However, as Biberfeld et al. have pointed out, there is a possibility that antibody-dependent lymphocytolysis and phytohemagglutinin (PHA)-induced cytolysis (T cell-mediated killing) both operate through the activation of a common cell type (132). It may therefore be concluded that some cells important for lymphocytolysis, in vitro, have a requirement for antibody attachment to the target cell. MacLennan (753) has suggested the term "cytotoxic B cell" for the non-glass-adherent, nonphagocytic mononuclear cells that kill target cells sensitized with IgG. He regards such cells as athymic in development and postulates that they do not release lymphokines. (It is now known that B cells *do* release certain lymphokines.) If this cytotoxic lymphocyte is indeed a B cell, then here again is evidence that, in the "normal" immune system, the functions of the B component (HI) and the T component (DH) are interdependent.

Antibodies reactive with purified protein derivative. Kostalia has followed guinea pigs immunized with 2.4 mg of heat-killed *M. tuberculosis* in adjuvant (Bayol F, 90%; Arlacel A, 10%) from 4 days to 1 year postimmunization. She examined the capacity of the serum immunoglobulins produced to (i) cause the hemagglutination of PPD-coated erythrocytes, (ii) effect the passive immune hemolysis of those antibody-coated erythrocytes (648), and (iii) render peritoneal exudate cells able to form rosettes with PPD-coated SRBC (macrophage-bound cytophilic antibody) (650). The results regarding cytophilic antibodies (on macrophages and in sera) indicated that they were present in small amounts in a few animals at the end of 1 month postimmunization but, at the end of 1 year, were present in a larger number of animals and in higher concentrations. (The possible contribution of these immunoglobulins to measurement of MIF in migration inhibition tests is considered under the section, MIF.) Hemagglutinating antibodies appeared as early as 5 days, whereas immediate reactions (4-h reactions, Arthus type, elicited in response to 10 μg of PPD) appeared in a few animals as early as 10 to 14 days. Tests for each of these responses were uniformly positive at 2, 4, 6, and 12 months postinfection. The highest agglutinin titers (1:5,120) were observed 28 and 35 days postsensitization. These persisted for 1 year. They were 2-mercaptoethanol sensitive and, so, could thereby be distinguished from 7S antibody (127).

Delayed Hypersensitivity, the Jones-Mote Reaction (Cutaneous Basophil Hypersensitivity), Contact Sensitivity, and Suppressor B Cells

When DH reactions are recorded here, they may well be either Jones-Mote reactions or typical DH, for until recently distinctions between the two were seldom made. Cutaneous basophil hypersensitivity (CBH; the Jones-Mote reaction [560]) is a transient dealyed skin reaction, occurring in guinea pigs sensitized with insoluble antigen-antibody complexes (in IFA) and with the contact allergen dinitrofluorobenzene (DNFB), in which the infiltrate contains large numbers of basophilic leukocytes in addition to the round cell component characteristic of DH (330, 977). Like classic DH, CBH reactions depend on lymphocyte function but are distinguished by a lack of induration. When cells from CBH-positive animals were transferred to nonimmune recipients, subsequent skin testing elicited small reactions (averaging 6.1 mm in diameter), which histologically were weak CBH reactions. The calculated number of basophils in the passive transfer was no more than 300,000 of 3×10^8 viable lymphoid cells. Passive

transfer of serum brought about no positive reactions in the recipients (see Askenase et al., just following). Dvorak et al. (330) have further pointed out that basophils in substantial numbers have been identified in contact allergy (in guinea pigs and in man), in hypersensitivity to vaccinia, and in allograft rejection, as well as in CBH reactions. They occur to a lesser extent in the "classic" DH that follows immunization with protein antigens in CFA. Their function in these various reactions is not clear. Stadecker and Leskowitz (1080) have found that skin reactions, elicited in normal guinea pigs in response to PHA, Con A, and pokeweed mitogen *but not* to the B cell mitogen, *E. coli* lipopolysaccharide, were rich in basophils. This has suggested to them that T cells release a factor chemotactic for basophils. Richerson has suggested a relation between CBH and passive cutaneous anaphylaxis (homocytotropic) antibody (976). Dvorak et al., however, have shown that circulating basophils from animals primed for CBH *lack* demonstrable specificity for sensitizing antigen. They feel that homocytotropic antibody has no obvious role in CBH. They conclude that CBH and DH have an analogous lymphocyte-mediated pathogenesis (329). Askenase et al. have reported the transfer *by serum* of CBH to keyhole limpet hemocyanin (KLH). Furthermore, they have shown that skin testing per se induces CBH. This important observation they have discussed in relation to interpretations given to specific immune reactivity following transfer of serum, cells, or cell extracts (56).

Recently, Turk et al. and Parker (1184) have defined two forms of DH in guinea pigs receiving ovalbumin. (i) In CFA, one form leads to a reaction that bears "a close similarity to the classical tuberculin reaction" (DH: skin reactivity developing within 7 days and reaching maximum intensity at 24 to 30 h post-testing, with persistence of inflammation after 48 to 72 h). (ii) In IFA, the other form is detectable 5 to 10 days after inoculation, reaches its maximum intensity between 24 and 30 h, and fades at ±48 h; it was defined by Turk as CBH. At 14 days this sensitization becomes an "Arthus or immediate-type" skin reactivity. The authors further established the fact that treatment of guinea pigs (or mice) with cyclophosphamide (CY), 300 mg/kg, "selectively depleted" lymphocytes from the lymph follicles, the corticomedullary junction in lymph nodes, and from analogous non-thymus-dependent areas of the spleen. This lowering in the populations of rapid-turnover lymphocytes (presumably B cells) was reflected in the immunized animal by lowered levels of IgG_1, homocytotropic antibody. When the responses were compared between control animals and CY-pretreated animals, both receiving ovalbumin *in IFA*, they were found to be clearly different (see Fig. 28). Those animals pretreated with CY (3 days before sensitization), and therefore experiencing a depletion in presumed B cells, developed typical DH reactions (as defined above). The control animals, on the other hand, exhibited a CBH type of response. Here, then, B cell elimination has enhanced DH. The authors offer evidence from a number of ancillary experiments to make it clear that suppressor B cells may regulate the activity of T cells. In fact, their report confirms and extends that of Neta and Salvin (875, 876).

Turk et al. also found that, in the B cell-depleted animals, the amount of basophil infiltration was markedly reduced. Thus, a so-called cell-mediated response is regulated by both T cells and B cells. The authors further showed that, in CY-treated animals sensitized to DNFB, there was a somewhat comparable shift from larger and longer-lived skin test reactions in the B cell-depleted animals (24 to 96 h) to less-intense and shorter-lived reactions in the control animals (24 to 48 h). One other

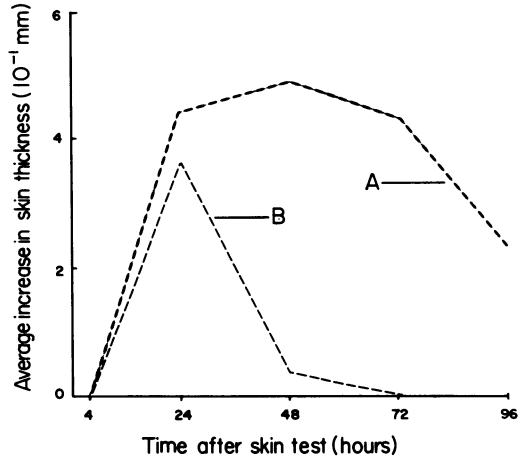


FIG. 28. *Average increase in skin thickness after skin testing with 100 μg of egg albumin in guinea pigs immunized with egg albumin in IFA 7 days earlier. (A) Reactions (DH) occurring in animals pretreated with cyclophosphamide (CY) 3 days prior to sensitization. (B) Reactions (CBH) occurring in untreated control animals. When untreated animals are immunized with egg albumin in CFA, they give a reaction essentially like that in curve (A) (DH), but when the protein antigen is administered with IFA the reaction of curve (B) (CBH) obtains. Thus, CY pretreatment modifies the CBH reaction expected with egg albumin in IFA, rendering it equivalent to the DH reaction normally obtained with egg albumin in CFA. Adapted from Turk et al. (1185).*

singular finding in this report was the fact that, in animals made tolerant to dinitrochlorobenzene, either by feeding or by intravenous dosages, tolerance could easily be broken in CY-treated animals. The authors conclude that the differences observed in classical DH, CBH, and contact sensitivity are reflections of degrees of interaction of effector (T) and suppressor (B) elements, particularly at the reaction site.

Delayed Hypersensitivity and Cell-Mediated Immunity

From the foregoing it is clear that the term cell-mediated immunity (CMI) is difficult for the beginning student since all immunity is cell mediated. Isolated immunoglobulin (humoral immunity) and isolated lymphokines (activation factors from T and B lymphocytes) are cell products and their action in the immune system must be understood at the cellular level. DH, commonly equated with CMI, is also poorly understood. The triad of the immune system, the B cell component (primarily immunoglobulin synthesis), the T cell component, and macrophages, interacts in the wake of inflammatory responses to control *chronic* infections. Limitations in understanding the behavior of this system are related to difficulties in dealing with each of its isolated components. We have, in the preceding section, given some gleanings regarding the behavior of the B cell component in mycobacterial infections. Cytophilic antibodies made by B cells could effect cytotoxic killing by macrophages. The level of antibody synthesized by B cells in response to certain antigens could be strongly influenced by T cells (see Katz and Benacerraf [602, 603]). Thus, helper T cells seem critical in the humoral immune response. There is a question as to whether or not some of them synthesize immunoglobulin (for a discussion of this point see reference 1221a). DH is thought not to occur in the absence of T cells. They have a primary but not exclusive role in DH. Some of the lymphokines attributed to them are also produced by B cells (e.g., in lymphocytes from BCG-immune animals, PPD induces interferon production [1218], MIF, and other products discussed below). Thus, Mackaness has suggested that the role of T cells in resistance to bacterial infections is as "molecular mediators" possibly responsible for the structural changes that characterize activated macrophages (752). (These T helper cells and cytotoxic T cells, KTC [effector cells], are thought to be separable on the basis of sedimentation velocity profiles [340].) Bloom has pointed up the difficulty in detecting specifically sensitized cells (the mediators of DH) by their ability to produce lymphokines. Whereas one can examine the ability of B cells to produce antibody (e.g, in the Jerne plaque assay), the amount of factors such as MIF/cell or interferon/cell is very little "and activity may not be detected even at small distances from target cells" (146). The function(s) associated with the T cell component of the immune system that can be well assessed in vitro is antigen-induced lymphocyte cytotoxicity, reviewed by Cerotini and Brunner (227). The prototype of the killer lymphocyte (cytotoxic lymphocyte, KTC), derived from antigen-reactive precursors, was a lymphocyte induced in graft-versus-host reaction, in which the specific inducing antigen occurred on allogeneic cells which themselves became the targets (cells) of lymphocyte killing. Killing reached completion within 1 h and was modulated by intracellular levels of cyclic adenosine monophosphate (AMP). Antibody directed against the surface antigens of the target cells protected the latter from KTC killing. This role of the KTC seems well adapted to the job of immune surveillance (206, 1152), as in the rejection of grafts and in the destruction of neoplastic cells. KTC seems not to be a cell designed for killing bacteria. Could its innate capacity for destroying foreign cells when their foreign-ness is due to subtle differences in surface antigens (e.g., HLA antigens) complicate DH reactions involving bacterial antigens; e.g., chicken RBC, which have been coated with PPD, are susceptible to cytolysis by lymphocytes from BCG-sensitized donors (919)? Will cells from tuberculin-positive individuals, coated with PPD during a skin test, be liable to killing by KTC and/or other K cells (920)?

The same sort of question can be asked regarding armed macrophages. Evans and Alexander (359) have shown that the killing specificity of macrophages involves their being "armed" against specific tumor cells [SL2(DBA/2) and TLX9(C57/B1) lymphomas]. Arming may be accomplished by (i) deriving macrophages from peritoneal exudate cells (PEc) of specifically immunized mice, (ii) arming in vitro by association of nonimmune macrophages with spleen cells from hyperimmune mice, or (iii) arming in vitro by exposure of nonimmune macrophages to cell-free supernatant obtained when spleen cells from immunized mice are cultured with specific antigen. All such macrophages, following incubation with specific antigen, undergo *activation* to killing of target cells. The killing follows contact of the target cell membranes with the macrophages. Phagocytosis occurs only after the target cell has begun to disintegrate. Macrophages from BCG-immunized animals kill neither type of tumor

cells. However, if such macrophages are incubated with PPD, they now are *activated* to kill either of the antigenically distinct tumor cells. A careful examination of the data in Table 7 reveals that immunization of animals with specific antigen (specific tumor cells) yields a macrophage armed to kill that particular cell and, further, that *incubation* of macrophage populations (taken 10 days after primary immunization) from any of several specifically hyperimmunized animals (*"armed macrophages"*) *with the specific immunizing antigen* will activate them to kill cells bearing other specific antigens upon their surfaces (Table 7). Thus, BCG "armed macrophages" can be *activated* in the presence of BCG to kill SL2 cells and TLX9 cells. They cannot be so activated by antigens other than the immunizing antigen. Will "armed macrophages" in a tuberculin-positive individual kill cells that have bound PPD? The macrophage-arming factor, which is released by lymphocytes, will undoubtedly turn out to be a cytokine such as MIF (358), and arming and activation will become synonymous with the enhanced-capacity macrophage discovered by Elberg (338) and rediscovered and expanded upon by Mackaness (751, 752). Germain et al. have confirmed the general findings of Evans and Alexander (359). For an assessment of tu-

TABLE 7. *Cytotoxicity of macrophages from immunized mice*[a]

CBA mice immunized with:	Macrophages incubated with[b]:	Cytotoxicity[c] at 48 h to:	
		SL2 (%)	TLX9 (%)
SL2 lymphoma (DBA/2)	No antigen	99	<10
TLX9 lymphoma (C57B1)	No antigen	<10	99
BCG	No antigen	<10	<10
SL2 lymphoma	SL2 cells	99	79
	TLX9	99	<10
	PPD	99	<10
TLX9 lymphoma	TLX9 cells	75	99
	SL2 cells	<10	99
	PPD	<10	99
BCG	PPD	92	84
	SL2 cells	<10	<10
	TLX9 cells	<10	<10

[a] Adapted from Evans and Alexander (359).

[b] PEc taken at 10 days after second immunization showed specific cytotoxicity. However, if a further dose of specific antigen was given and macrophages were collected 24 to 72 h thereafter, those macrophages killed both SL2 and TLX9 cells. This was the case for macrophages from either SL2-, TLX9-, or BCG-immune mice. Ten days after the third immunization, however, the activated macrophages had disappeared, and the macrophages found in the PEc possessed immunologically specific cytotoxicity shown above. For further details, see text.

[c] Cytotoxicity expressed as percent growth inhibition in relation to growth in normal or control CBA cultures.

mor cell inhibition, they have measured the inhibition of DNA synthesis (IDS), as indicated by the uptake of [^{3}H]T by tumor cells in the presence and absence of effector cells (presumably macrophages):

$$\frac{\text{cpm (effector + tumor)} - \text{cpm (effector alone)}}{\text{cpm (tumor alone)}} \times 100$$

and compared these results with those obtained by measuring ^{51}Cr release (1264a). When these authors (427a) examined that fraction of the PEc that was non-glass-adherent, they discovered another population of lymphoid cells capable of specifically lysing tumor cells. Although the details of their characterization of the cells were necessarily limited, those cells are probably most akin to the natural killer lymphocytes (NK) of Herberman (503a, 503b) and of Kiessling (613a, 613b) (see following).

Wolfe et al. (1274a) have recently reported the amplification in PE of a mouse cell type capable of lysing both syngeneic EL-4 thymoma and allogenic P815-X2 mastocytoma target cells following intraperitoneal (i.p.) infection of mice with BCG. The cells were non-glass (polystyrene, in this case)-adherent. Their killing activity was enhanced rather than diminished when the population was exposed to 200 mg of carbonyl iron per 2×10^7 cells and subjected to a magnetic field that removed 60% of the population. (i) This would seem to mitigate against the idea that the cells are macrophages. (ii) Treatment of the population with anti-Θ serum and complement (under conditions where identical treatment removed 91% of the T-cell mediated [KTC] cytotoxic activity of spleen populations from C57BL/6 mice immunized with P815 [DBA/2] alloantigen) failed to lower the lytic activity, thus suggesting that lytic activity could not be ascribed to T cells. (iii) When the numbers of the population having Ig on their surfaces were lowered from 12 to 2.4%, there was no effect on lytic activity. Thus, the B cells in the population seemed not involved in the lysis under study. (iv) Fc-receptor-requiring, trypsin-resistant, antibody-dependent cytolytic activity was also ruled out. The authors conclude that they were dealing with a "natural killer" cell previously described by Herberman (503a, 503b) and by Kiessling (613a, 613b). When PEc were raised with thioglycolate or proteose peptone, no lytic activity for P815-X2 was found. Following i.p. immunization with BCG, cytolytic cells appeared. The authors have suggested that BCG might: (i) induce the cytotoxic population *ab initio*; (ii) cause the expansion of clones of cytotoxic cells existing in undetectable numbers; (iii) recruit cytotoxic

cells to the peritoneal cavity from other areas; or (iv) activate an exudate cell population to cytotoxicity.

Humoral Immunity, Delayed Hypersensitivity, and Cell-Mediated Immunity in Mycobacterial Infections

In three solicited editorials on "Delayed Hypersensitivity and Immunity in Tuberculosis" appearing in the *American Review of Respiratory Diseases*, the following conclusions were expressed: (i) " . . . reactions of tuberculin hypersensitivity play little, if any, role in acquired immunity to tuberculosis" (1304); (ii) at present the conditions and reagents employed for studying DH on the one hand (soluble antigens) and CMI on the other (living bacteria) sometimes suggest a correlation and sometimes do not. "More extensive standardization of antigens and assay procedures must be developed. With this additional information and procedures, the data that now seem contradictory may actually be complementary" (1016). (iii) "The evidence for the essential indivisibility of DTH and immunity is circumstantial and relies heavily on the strong association between the two phenomena." The author concludes from a mass of "disparate data that lymphokine production, blast transformation, DTH, and immunity are predominantly, if not exclusively, T cell functions. Thus the T cells are the common denominator that link DTH, immunity, and their *in vitro* analogs." He further suggests that to establish a separation between DH and immunity an immunizing agent should be found which is nonallergenic, admonishing that "no claim should be made for such agents without exhaustively testing several parameters of cellular hypersensitivity so as to ensure that the materials are truly nonsensitizing" (711).

DH, then, appears to be a necessary adjunct to the interactions that lead to CMI. It is destructive for the animal and is thought to contribute to the development of the disease process in tuberculosis. In the experiments of Turk, cells associated with the HI component of the immune system (presumed B cells) exerted a suppressive effect on the extent of DH inducible by specific elicitins. Presumably, this suppressive effect would be proportional to the number of suppressor cells available. If that is true, then the HI component of the immune system, including any specifically amplified population thereof, would be important in recovery from tuberculosis. In this regard the suppressor effect of B cells would have a benefit for the host comparable, to a degree, to that reputedly associated with desensitization (549, 1291).

Elsewhere, we have pointed out that the elicitin, PPD-S, is nonpriming per se but, when given to an animal along with poly(A)·poly(U), DH develops. Since this cannot happen without the release of lymphokines, it necessarily involves the activation of macrophages via MIF, etc. Since many cell types including B and T lymphocytes produce MIF, an as yet limitedly characterized cytokine (see Cohen in reference 885), since B cells exert a suppressor action in DH, and since B cells are linearly related to antibody-producing plasma cells, HI and DH would appear to be more, not less, inextricably related than ever. For a general discussion of DH in relation to CMI, see McCluskey and Cohen (802a).

Lymphocytes, Macrophages, and Mycobacteriostasis

Dienes and Mallory showed that *new lesions*, produced by injecting *M. tuberculosis* into *tuberculous* (*already hypersensitive*) guinea pigs, were characterized for the first 24 to 48 h by a marked accumulation of granulocytes, congestion, and serous exudation, a reaction suggestive of any pyogenic infection. However, with great regularity, beginning on day 3 and becoming well marked on day 4, "collections of large mononuclear phagocytes begin to appear as cuffs about blood vessels and nerves, to infiltrate the stroma first at some distance from organisms and leucocytes but gradually to condense about them to form a wall several cells thick" (305). It is an old story that these cuffs consist largely of macrophages and lymphocytes (see section on Granuloma).

A role for armed and activated macrophages in resistance to tuberculous infection has come to be accepted (140, 740, 1048), and similar macrophage involvement has been demonstrated in infections with *Salmonella typhi* (1011), *Listeria monocytogenes* (820), and *Brucella abortus* (517), as well as certain protozoa and viruses (397). Since Elberg's observation in 1957 (338), it has become clear that macrophages from a specifically infected animal, on subsequent challenge, show an enhanced capacity to cope with the original infecting organism, as well as any one of several unrelated organisms but, to date, the mechanism by which the bacteria are killed is not known (885).

It is a long time since Rich and Lewis made the observation that cells from spleens and lymph nodes of tuberculin-sensitive animals were inhibited in their migration by tuberculin (974). The present textbook picture of the specificity of migration inhibition (146, 335) dates from the experiments of George and Vaughan

(427), in which the migration of peritoneal exudate cells was shown to be inhibited following the addition of tuberculin or egg albumin, and the development of sensitivity to inhibition paralleled a development of delayed skin reactivity to egg albumin or tuberculin (see review by David [290]). T lymphocytes derived from animals rendered tuberculin sensitive by vaccination with BCG respond to tuberculin by releasing (among other lymphokines [324]) a factor that inhibits the migration of macrophages (migration inhibition factor, MIF) from capillary tubes (147). Macrophages from immunized as well as nonimmunized animals nonspecifically respond to MIF. MIF can be produced by nondividing lymphocytes (987). The activities of MIF may be complex (871) and are probably affected by the levels of cyclic AMP in macrophages (643) at the time MIF reaches them. Although the production of lymphokines such as MIF has at first seemed to be a specific attribute of T cells, this apparently is not so: human B cells from tuberculin-sensitive (as well as from *Candida*-sensitive and streptokinase-streptodornase–sensitive) donors produce MIF upon exposure to their respective elicitins, e.g., PPD. Whereas T cells, after exposure to PPD, undergo proliferation, B cells do not. Only T cells, following exposure to specific antigen, produced the lymphokine, lymphocyte mitogenic factor (LMF). This LMF induces [^{3}H]T incorporation into both T and B cells obtained from donors lacking sensitivity to the antigens used to elicit the factor. Thus, both MIF and LMF, although induced by specific antigens, act upon cells from unprimed animals (988). These findings indicate that, for the most part, although MIF production is associated with DH, it is not associated with, or indicative of, activity by any one cell type. Further, the T component and the B component of the immune system must be composed of a number of subtypes. It is evident from the above that, in experiments designed to determine the portion of tuberculin that can interact with T cells or B cells, one needs not only chemically well-characterized mycobacterial products, but also relatively homogeneous populations of T and B lymphocytes. Previous experiments showing the induction of blastogenesis in lymphocytes by nondialyzable proteins from "tuberculin" (92a, 233, 544) will take on more meaning when repeated with better-characterized populations of lymphocytes.

Soluble factors capable of inactivating, in vitro, *S. faecalis*, *S. aureus*, *P. aeruginosa*, and *Candida albicans* and of suppressing hematopoiesis in bone marrow explants were demonstrated in the sera of mice initially infected intravenously (i.v.) with BCG and challenged i.v. 3 weeks later with OT (1016a). Flower et al. have described a soluble factor, formed by lymphocytes in response to streptococcal filtrate or phytohemagglutinin (PHA), which could reduce the viability of *E. coli* grown under special conditions (381a). The implication in these papers is that some cytokines may act directly on bacteria.

Mackaness and his colleagues suggested an antigen-specific role for an immunologically committed lymphocyte in inducing, in vivo, *enhanced* macrophage resistance to homologous challenge (751). Patterson and Youmans have shown that splenic lymphocytes from mice immunized with living $H_{37}Ra$ released (a) substance(s) which inhibited the multiplication of $H_{37}Rv$ in peritoneal macrophages from normal mice (910). Krahenbuhl and Remington, using *Toxoplasma gondii* and *L. monocytogenes*, succeeded in demonstrating that lymphocytes from specifically sensitized inbred strain XIII guinea pigs could induce in normal peritoneal macrophages resistance to heterologous ("nonspecific") as well as homologous challenge (659). Youmans and associates, in a series of papers, have examined products of lymphocytes that activate macrophages to enhanced bacteriostasis. They conclude that a growth inhibitory factor (GIF) is formed in lymphocytes grown in the presence of any one of several bacteria (e.g., *L. monocytogenes*, *M. tuberculosis*, etc.). GIF can be induced by bacteria added to lymphocytes from immunized as well as nonimmunized mice (620). When isolated preparations of GIF are added to macrophages there is an arrest of the intracellular growth of the infecting organism. GIF is said to be separable from MIF (618). GIF is neither species nor genus specific, for GIF induced by $H_{37}Ra$ or *L. monocytogenes* is effective in blocking the intracellular growth of both $H_{37}Rv$ and *L. monocytogenes* (559, 620). Although GIF is effective against *intracellular* $H_{37}Ra$ and *L. monocytogenes*, it is without effect on these microbes grown extracellularly (619). Thus, GIF is probably an inducer of a factor of growth inhibition in macrophages. Lymphocytes could be stimulated to produce GIF in vitro. While Con A stimulated the production of GIF, PHA did not (620). GIF seems, then, to be produced in response to a class of mitogens rather than specific organisms. Thus, $H_{37}Ra$, *L. monocytogenes*, and Con A all stimulate the production of GIF in normal as well as "immune" lymphocytes. It remains to be shown that GIF is not equivalent to the macrophage activation factor (latent period of 3 days for development following antigenic stimulation)

of Nathan et al. (870) or even MIF. GIF is probably a glycoprotein. It is of low molecular weight (see also Kühner and David [674]), is sensitive to pH 2.0 (see also Salvin et al. [1017]), and is sensitive to chymotrypsin (see also [674]). It is nondialyzable, and its synthesis is blocked by inhibitors of protein synthesis (209, 210, 211). Salvin et al. have shown, with regard to the difficulties involved in sorting low-molecular-weight activation factors, how similar MIF and interferon are (1017, 1305). Whether the primary effect of GIF on macrophages is at the level of macrophage lysosomal activity remains to be decided.

Hart and Armstrong (494) have recently reported a comparative study of phagolysosome (fusion of secondary lysosomes) formation in cultured macrophages in response to $H_{37}Rv$ and $H_{37}Ra$. It was not possible to carry out the experiment in such a way that both bacterial populations were treated identically. In week 1, there was a drop in the number of viable $H_{37}Ra$ as compared with $H_{37}Rv$, and this roughly correlated with a higher level of fusion between intact organism-containing phagosomes and ferritin-prelabeled lysosomes. Later, the numbers of $H_{37}Ra$ did increase. From this trend the authors conclude that $H_{37}Ra$ is an *attenuated* but not a truly avirulent strain. Hart and Armstrong (51) have found that pretreatment of tubercle bacilli ($H_{37}Rv$) with specific antiserum prior to their ingestion by macrophages led to fusion of bacilli-laden phagosomes with ferritin-labeled lysosomes as quantitatively assessed using electron microscopy. Bacterial viability studies indicated that serum pretreatment was itself not bactericidal. No difference in the intracellular growth rates of serum-treated and untreated bacilli was found. Electron microscopic monitoring of this material revealed that bacilli remained intact and multiplied, both in phagolysosomes and in unfused phagosomes. It was concluded that turning on of "phagosome-lysosome fusion in *normal* macrophages did not influence the outcome of infection with virulent *M. tuberculosis*; lysosome contents . . . failed to exercise an antibacterial effect on this organism. Nevertheless the possibility remains that the lysosomes of specific immune macrophages have antituberculous potentiality. In that case the experimental 'turning on or off' of fusion could be a decisive factor in the outcome of a virulent challenge. Should it not be, the antibacterial capabilities of immune cells would need to be ascribed to factors other than lysosomal attack, the latter being essentially for disposal of dead organisms." This scholarly and provocative paper contains a useful review of the effects of serum on the outcome of macrophage-"parasite" interaction, which differs from system to system. For example, in the case of *Toxoplasma gondii*, reported experiments of Jones et al. showed that, whereas *T. gondii* could fluorish in the phagosomes of macrophages in the absence of antiserum, following pretreatment with antibody there was ample evidence provided by electron micrographic monitoring of an increase in phagolysosomes enclosing organisms in an obvious state of degeneration. See also Dannenberg et al. (281), Kanai and Kondo (574), and the section on Granuloma Formation. Whether GIF brings on the production of the bacteriostatic heptane-soluble fatty acids called HEF by Kochan and Golden (626; see also references 337, 496, 638) or the induction of an enzyme that cleaves such FA from more complex mycobacterial FA is intriguing, since the sensitivity of certain mycobacteria to long-chain fatty acids has long been known (321).

(Recently, Hahn [485] and Hahn and Bierther [486] have shown that the polymer dextran sulfate 500 [DS 500], in doses of 50 mg/kg of mouse, brings about the autolysis of a number of macrophages, thus limiting the amount of phagocytosis the macrophage population can effect. In experimental infections in mice with *L. monocytogenes*, DS 500 administered anytime [see reference 158] during the first 3 days led to an immediate increase in bacterial counts and, in many cases, death. Additional experiments indicated that, in the presence of DS 500, mice were unable to develop antilisterial immunity following either active immunization or passive immunization through the administration of spleen cells from *Listeria*-immune donors [485, see also references 401 and 1051]. These data offer circumstantial evidence to support the general thesis that the inactivation of macrophages would result in effective impairment of cell-mediated immunity [CMI]. According to Bonventre [personal communication], DS 500 also induces a drop in polymorphonuclear cells in mice. Thus, DS 500 appears to inhibit not one but two classes of phagocytic cells.)

Migration Inhibition Factor and Delayed Hypersensitivity in Immunized Guinea Pigs

Kostiala (648) found that delayed skin reactivity (10 μg of PPD per ml, eliciting dose; 24-h reading) developed a little bit earlier (4 to 6 days, depending upon peculiarities of the animals) than migration inhibition responses to 25 μg of PPD per ml (7 to 8 days). She subsequently showed that, whereas the stimulation of the formation of MIF by PPD required 25 μg of PPD per ml at 7 days, only 0.01 μg/ml was

required at 6 months. Kostiala reasoned that this increased sensitivity to stimulation may have resulted from (i) increasing affinity of the lymphocyte receptors for PPD, (ii) the activation of lymphocytes by antigen-antibody complexes, and/or (iii) the presence of cytophilic antibody on the surface of macrophages (649). The capacity to bring about migration inhibition was much more marked at the end of 1 year than at the end of 1 month. Passively sensitized (with antibody) guinea pig sera showed little change in effect on migration of macrophages. Since MIF is generally measured as an effect on cells rather than as a specific activity, there is always the possibility that more than one substance may inhibit the migration of macrophages.

Development of Autoantibodies in Mycobacterial Infections

It has been known for over 50 years that sera from patients with chronic pulmonary tuberculosis often have the capacity to precipitate saline extracts of normal human lung, liver, etc. Thewaini Ali and Oakley have reviewed the literature on the association of the production of autoantibodies with chronic infections as an introduction to their experiments, which establish unquestionably that the production of autoantibodies in the host is not an uncommon event in rabbits chronically infected with *Pasteurella pseudotuberculosis* and *M. tuberculosis* (1150). Sera from uninfected animals or sera taken from animals prior to infection contained no autoantibodies. Following infection, about one-quarter of the animals developed antibodies against antigens from rabbit liver, kidney, spleen, lymph-gland, lung, and heart. Rabbits immunized with phenol-sterilized pseudotuberculous rabbit organ homogenate in IFA developed autoantibodies faster than rabbits immunized with normal rabbit organs in IFA. Sera containing high titers of autoantibody were markedly cytotoxic for liver, spleen, and kidney cells in culture but not for lymphocytes or macrophages.

The various experiments of these authors support the thesis that developing infections alter normal high-molecular-weight tissue components in such a way as to render them antigenic, with the result that antibodies produced against these modified tissues react with normal tissue components. It was pointed out some years ago that all autoantibodies do not produce deleterious effects and that Ehrlich's concept of "horror autotoxicus" being generated by such antibodies could only partially be justified (675a). Examples of innocuous autoantibodies are those involved in clearing up cellular debris or acting as "transporteurs" for animal cells, or both, and have been discussed in some detail by Grabar (461a). There exist a number of resumes of autoimmune disease and criteria required for establishing autoimmune etiologies (e.g., 335, p. 585–588, and the laboratory tests for autoimmune diseases by Nakamura et al. [867a]). Information on the classes of antibody molecules involved in autoimmune processes is often lacking in reviews of such disorders as thyroiditis (1013a, 1053a). Recent papers do attempt to define the class of antibody being studied. For example, autoantibodies acting as complement-dependent, serum-demyelating factor in autoimmune encephalomyelitis in the guinea pig have been shown to be IgG_2 and neither IgG_1 nor IgM (697a). An indirect identification by antiglobulin consumption tests of incomplete platelet autoantibodies in idiopathic thrombocytic purpura, systemic lupus erythematosis, and autoimmune hemolytic anemia indicated the presence of IgG and the absence of IgM in 31 of 33 cases (359a). Antibodies that block the uptake of vitamin B_{12} (blocking antibodies) by gastric intrinsic factor have been shown to be IgG (1211b), as have some of the antiacetylcholine receptor factors in sera of patients suffering from myasthenia gravis (834a). The autoantibodies most characteristic of rheumatoid arthritis (RA) are anti-IgG antibodies or rheumatoid factors (RF): IgG RF and IgM RF. Complexes of IgG·IgG RF apparently play an important role in the pathogenesis of RA (1310a). The pros and cons of a role for IgM RF in rheumatoid arthritis have recently been reviewed by Wager (1211a). An anticoagulant activity (a presumed antiprothrombinase) found in a case of chronic lymphocytic leukemia was classed as an IgM antibody (521a). Although the role of immunoglobulins has long been considered in autoimmune disorders, only more recently has the interaction of T and B lymphocytes in such conditions been investigated (e.g., reference 261a), and the use of the induction of specific immune unresponsiveness been considered as a means of preventing deleterious autoimmune effects (42a). Studies of autoimmune reactions as concomitants of mycobacterial infections are few (1150, and reviewed in [1184]) and rarely have they defined antibody activities in terms of classes of immunoglobulin (853a).

Adjuvant Effects on the Cellular Components of the Immune System

In 1924, Lewis and Loomis (721) made acceptable an old idea about the general refractoriness to infection of people with tuberculosis by making the discovery that tuberculous infec-

tion raises the "allergic irritability" of animals. They (722) showed that there is a marked enhancement in antibody formation by guinea pigs injected with living tubercle bacilli in the peritoneal cavity a few days prior to receiving antigens such as SRBC or killed *S. typhi*. Dienes confirmed and pointed up the *general* application of these findings by showing that induced hypersensitivity to egg albumin and timothy pollen differed in the tuberculous and the nontuberculous guinea pig (302, 303, 304). When Freund tried to repeat Dienes' experiments, using *killed* tubercle bacilli, he had no success. Out of this *no success* eventually came the discovery that *dead M. tuberculosis in water-in-oil emulsions* (mannide monooleate, 1.5 parts, to paraffin oil, 8.5 parts, vol/vol) *behave like living M. tuberculosis* in enhancing antibody production to cointroduced antigens, as well as inducing specific DH to tuberculin (398; see also BCG, just following). [As pointed out in the section on Elicitins, the best example of a chemically defined adjuvant for use in sensitizing animals to PPD-S is the polynucleotide poly(A)·poly(U).]

Freund and his co-workers found that other mycobacteria (e.g., *M. smegmatis* [*butyricum*], as well as *Nocardia asteroides*) served as good adjuvants. Freund subsequently showed that lipoidal extracts from *N. asteroides* were also effective as adjuvant. Animals sensitized with *N. asteroides* gave positive skin reactions to OT (399). It has been presumed that water-in-oil emulsion provides a depot from which antigen can be slowly released. Such slow release has in common with chronic infection the protracted availability of antigen. Oil droplets containing antigen have been found in cervical lymph nodes 3 weeks after the injection of IFA (1251). The enhancement of antibody production by CFA is correlated with the formation of a granuloma at the site of injection and hyperplasia in the regional lymph nodes (375). It has been suggested that this hyperplasia occurs in areas of T cell proliferation (335; see also [25, 317, 1144]). Although the specific mycobacterial product(s) functioning in this stimulation is (are) not known, the mycobacterial cell wall component, wax D, was early used as a substitute for mycobacterium in CFA (1252; see also [243, 812]). Freund long ago showed that, on a weight basis, whole mycobacterium was 100 times more effective in CFA than wax D in stimulating, for example, DH to tuberculin (400).

Donald and Pound have shown that a single i.v. injection into rabbits of a "hard wax" prepared from *M. tuberculosis* caused an overall decrease in the circulating (blood) mononuclear cells for the first 8 h postinjection, followed by an increase between 24 and 48 h. The number of reticuloendothelial cells in spleen, liver, and lung that were able to incorporate [^{3}H]T into their nuclei increased after 24 h. Tritium-tagged monocytes in the lungs were related to developing miliary granulomas. A single injection of lipid into the footpad caused intense hyperplasia of the reticuloendothelial cells (REC) of the sinusoids of the draining lymph nodes. The REC hyperplasia reached a peak at 36 h and lasted until 72 h. Multiple injections of lipid caused a marked increase in the number of REC in the spleen and liver and a doubling in the weight of the spleen (309). The results of these experiments are in keeping with findings regarding migration and association of cells of REC in CFA-stimulated animals. For example, Taub et al. (1144) and Taub and Gershon (1143) have shown that in the adjuvant-stimulated node the early increase in cells is due to the *arrival* of cells, an event which occurs before the increase in mitoses. This arrival and sequestering of lymphoid cells within an antigen-stimulated lymphoid organ did not occur "in T-cell-depleted" animals (thymectomized, irradiated, and bone marrow reconstituted). Frost and Lance (408) have proposed that the capacity to bring about the sequestration of lymphocytes in lymphoid organs ("lymphocyte trapping") is an important but not exclusive property of adjuvants. Adjuvant-induced trapping (e.g., CFA) lasts over an extended period of time. Prior sensitization of animals potentiates trapping. Circumstantial evidence points to the macrophage as a cell central to the phenomenon.

The effects of adjuvants at the level of individual types of cells (e.g., macrophages, T lymphocytes, and B lymphocytes) has recently been reviewed by Allison and others (1280).

It appears that adjuvants and antigens, fixed to macrophages, are a part of an important first step in successful antibody formation. The transfer of macrophages with associated antigen and adjuvant to syngeneic mice results in increased antibody formation over that found in mice receiving macrophages and antigen only. Treatment of lymphocytes with adjuvant prior to using them for reconstituting irradiated recipients was without effect on the immune response (1079). The macrophage-associated antigen and/or adjuvant is probably located on the plasma membrane. This is suggested by the fact that treatment with trypsin or with antibody specific for the antigen results in an evident depression of the immune response that follows in the recipient animal (1192). Macrophage-plasma-membrane-bound antigen has

been termed a "superantigen" (1213a).

It has already been noted above that the "lymphocyte trapping" induced by adjuvant is diminished or does not occur in T cell-limited animals. Unanue (1191) has found that doses of beryllium sulfate which normally bring about a 10-fold increase in the immune response of mice to certain doses of keyhole limpet hemocyanin (KLH) have no enhancing effect on the immune response of mice that had been thymectomized, irradiated, and reconstituted with bone marrow cells. Additional evidence for a key role of T lymphocytes in the effectiveness of certain other adjuvants comes from experiments of Allison and Davies (25) and of Cone and Johnson (257). The latter investigators found that poly(A)·poly(U), classed as a T cell-oriented adjuvant by Allison (24), gives its fullest adjuvant effect in the presence of critical numbers of T cells but not under conditions where T cells are in excess. Allison has indicated that macrophages are necessary in the poly(A)·poly(U) augmentation of immune responses (antibody synthesis) in mixed lymphocyte cultures and that elimination of T cells through the action of anti-θ serum and complement results in an inhibition of overall thymidine incorporation (24). Friedman et al. (402) have found that poly(A)·poly(U) increased the incorporation of thymidine in lymphocytes from tuberculin-positive subjects when those lymphocytes were exposed in vitro to tuberculin.

Mishell and Dutton (829) devised a system for the in vitro synthesis of antibody by mouse spleen cells. Using heterologous erythrocytes as antigen, they could elicit a primary antibody response in cultured spleen cells from normal mice. The number of spleen cells making antibody could be measured by the hemolytic plaque assay. This system lends itself to a study of the influence upon antibody production of cell types and their products. Thus, Dutton and his associates have found a soluble product from T cells, either stimulated in the mixed lymphocyte reaction (83, 730) or in response to antigen, which can replace T cells in augmenting the primary in vitro response manifested by spleen cells exposed to antigen (327). Maillard and Bloom (763) have employed a similar system for examining the in vitro primary response of spleen cells derived from animals primed with *B. pertussis* and *M. tuberculosis*. When these cells in culture were exposed to a third unrelated antigen, SRBC, they produced antibody. The levels of antibody were much enhanced when either PPD or *B. pertussis* was added along with the SRBC. Supernatants derived from cell cultures after the exposure of adjuvant-primed cells to adjuvant *in vitro* contained a factor that enhanced the immune response of normal spleen cells to SRBC. Treatment of the cells with anti-θ serum and complement yielded suspensions whose supernatants failed to stimulate the primary response of spleen cells. Some T cells, then, appear to respond to such adjuvants as CFA and mycobacteria by the production of a soluble factor(s) which act(s) upon antibody-synthesizing cells (B cells) and, perhaps, macrophages.

CFA and antigenic competition. Taussig has shown that CFA can alter the outcome of an antibody response. When mice were immunized with mixtures of gamma globulin (rabbit) and albumin (rabbit), about 95% of the antibody was found to be anti-globulin, and 5% was anti-albumin. (The discovery of such antigenic competition has been ascribed to Michaelis in a comprehensive review by Taussig [1145].) When similar stocks of mice were immunized using CFA combined with albumin and globulin, the proportions were significantly altered: 40% was anti-globulin, and 60% was anti-albumin. Taussig has suggested the following general explanation for these results: "(a) CFA enhances antibody responses by its action on cooperating T cell function; (b) this increased T cell activity can in turn lead to the ability of an antigen to compete successfully against other antigens administered at the same time (antigenic competition); and (c) as a result, a change in specificity of the immune response may occur, compared with the response to the same antigens given without adjuvant" (1146).

CFA and the depression of DH and HI. Asherson and Allwood have found that the administration of CFA to guinea pigs 10 days prior to immunization with bovine gamma globulin (BGG) in CFA resulted in depression 14 days later in the mean diameters of skin reactions obtained in response to BGG (mean diameters were 22.2 mm in animals receiving no pretreatment and 18.0 mm in pretreated animals). Similar experiments employing HSA gave a depression from 23.2 to 6.0 mm; arsanil-*N*-acetyltyrosine gave 12.6 to 7.1 mm of depression. *Propionibacterium parvum* adjuvant behaved similarly. Further, pretreatment with CFA depressed DH induced by *P. parvum* adjuvant (with the antigen), and the converse appeared to be true: definite depression occurred in one of two experiments. Pretreatment also depressed the reactions obtained in contact sensitivity experiments in mice with picryl chloride (pretreatment in footpads; test measured mean increments in ear thickness at 24 h). Four-hour indurations (Arthus) in guinea pigs were also depressed by pretreatment with CFA. Pretreatment with IFA or with silica did not lead to a

depression in DH or HI (55). The authors have tested two general hypotheses regarding the mechanism(s) by which pretreatment with CFA depresses the DH: (i) pretreatment depresses the inflammatory responses that are essential for DH skin reactions, and (ii) pretreatment interferes with induction, maintenance, or performance of those specifically sensitized cells concerned with DH. Experiments were carried out to determine the effect of pretreatment on responses in (i) animals passively immunized with sera from hyperimmune animals and (ii) others passively immunized with peritoneal exudate cells (PEc) from immune animals *as well as* the response of normal animals *passively* immunized with PEc from CFA-pretreated and immunized animals (pretreated at day −10; immunized at day 1 and PEc harvested at day 14). Suitable control animals were included. It was found that pretreatment of guinea pigs with CFA depressed passively transferred 4-h (Arthus) skin reactions. This was interpreted as an effect on the inflammatory response. Pretreatment also depressed passively transferred 24-h DH skin reactions. Further, PEc from guinea pigs treated and subsequently immunized with BGG in CFA showed a depressed ability to transfer DH. This finding that CFA reduced DH by depressing inflammatory responses and the functioning of some cells of the immune system (26) may apply generally to antibody responses, as shown by the delay of immune-induced regression of hepatoma in guinea pigs after pretreatment with CFA (808). These effects of pretreatment with CFA probably represent the generation of suppressor T cells and the effect their presence adds to a continually changing immune response. As Asherson and Zembala (in 318, p. 158) have said: "each immune response is likely to favor certain other immune responses and to decrease the chance of others occurring."

CFA, PPD, and tuberculin anergy. Mycobacteria and mycobacterial products such as PPD are known to sometimes induce anergic states. Such depressed DH responses arise during some mycobacterial (973 [p. 447], 718, 780) and some viral infections (189, 579), during certain cancerous states (1095), during infection with *B. pertussis* (930), and during closely sequenced retesting of tuberculin-positive subjects with PPD (1149). Thestrup-Pedersen has examined the behavior of the lymphocytes of tuberculin-positive subjects showing a depressed skin response after testing with PPD and found a significant depression in the blastogenic response of such lymphocytes following exposure to PPD and to PHA. This transient suppression of DH in response to small amounts of PPD (0.02 μg) occurs in normal individuals and does not seem related to circulating antibody (1148). It may well relate to the immunosuppressive factor(s) found to be released by PPD-stimulated dividing lymphocytes from BCG-immunized animals by Outterbridge and Lepper and regarded by them as antigen-antibody complexes (900). These effects of CFA and PPD are aspects of immunological tolerance, a condition tied into the intricate interactions of the T and B components of the immune system. Diena et al. have shown that, during massive tuberculous infection, there is a shutoff in antibody synthesis. In animals moderately infected, there was evidence that a sustained antibody response required sustained input of antigen (301). In a study by Zeitz and co-workers (1309a), 6 of 458 patients with active tuberculosis were anergic by skin test at the time of diagnosis. In five, no cause for anergy could be identified. All five had precipitating antibodies to tuberculoprotein, and their lymphocytes responded poorly in culture to PHA and not at all to tuberculoprotein (PPD). During treatment, humoral antibody titers dropped, skin tests became positive, and lymphocyte reactivity in culture approached that of positive control subjects. Some of the possible mechanisms underlying the turning off of the immune system, with resultant immunological unresponsiveness, have recently been the subject of extensive discussions on immunological tolerance edited by Katz and Benacerraf (603) and, more recently, the papers on immunological tolerance edited for the *British Medical Bulletin* by Dresser (318). These include considerations of the effects of suppressor T and B lymphocytes, of "blocking" antibodies, anti-idiotype antibodies, and soluble immune complexes.

Intramacrophagic phospholipase A and adjuvants. Munder and Modolell (862) have found associated with macrophages from adjuvant-stimulated animals an increased formation of lysophosphatides. This they showed to be the result of the action of an intramacrophagic phospholipase A that seemed stimulable by adjuvants. The major lysophosphatide produced was lysolecithin. Lysolecithin itself turned out to be a good adjuvant. In experiments designed to test the effect of adjuvants upon the production of lysophosphatides by macrophages in vitro, the following protocol was employed for producing PEc: macrophages were produced by the i.p. injection of 2 mg of lysolecithin (1-acylglycero-3-phosphorylcholine) in saline into mice, which were subsequently sacrificed; their PEc were harvested, washed, and incubated

with [1-^{14}C]oleic acid (2 nmol per 2 × 10^7 PEc). Under these conditions [1-^{14}C]oleic acid was incorporated into cellular phospholipids and lipids as follows: 0.5 to 1% lysolecithin, 4 to 6% sphingomyelin, 55 to 60% lecithin, 8 to 12% cephalin, 2 to 4% oleic acid, and 15 to 20% neutral lipids. When 25 × 10^6 labeled PEc were incubated with adjuvant, e.g., *Propionibacterium parvum*, rapid degradation of labeled cellular lecithin occurred within the 1st h, with a concomitant accumulation of [1-^{14}C]oleic acid (863). This remarkable induction of intramacrophagic phospholipase A was shown to be effected by CFA (as well as each of its components given separately), by retinol (vitamin A), *P. parvum*, *B. pertussis*, *Salmonella minnesota* R 595, saponin, bentonite, $Al(OH)_3$, Al_2O_3, $BeSO_4$, and $Ca_3(PO_4)_2$, but not by endotoxin or "*Staphylococcus albus*." Thus, both T-oriented and B-oriented adjuvants (24) produced the effect in vitro upon PEc prelabeled with [1-^{14}C]-oleic acid. Exogenous lysolecithin acts as an adjuvant in vivo. The authors showed that the injection of 50 to 2,000 μg of lysolecithin (and some of its analogues) prior to antigen (BGG, BSA, and SRBC) produced a 10- to 100-fold increase in antibody titers in mice, as measured by hemagglutination, plaque-forming cells, antigen elimination, or antigen-binding capacity (862). These authors report experiments of Westphal et al., which showed the adjuvant effect on a weight basis to have been even greater when nonmetabolizable analogues of lysolecithin were given.

It is encouraging to think that some of the disparate molecular species which act as adjuvants may do so principally by their common ability to induce similar responses in macrophages. It may be worth noting that most adjuvants are administered with mineral oil. Even the adjuvant action of poly(A)·poly(U) was established using an oil vehicle (218, 219). Lysolecithin used for priming mice in the experiments reported above was given in saline. With regard to adjuvants that do not include an oil vehicle, the authors refer to Dresser's finding that free FAs show some adjuvant effect. For example, oleic acid plus BGG was a more effective combination than mineral oil and BGG (316).

Adjuvant disease. In rats inoculated with CFA, there occurs (in 10 to 16 days) an arthritis that is a part of a generalized disease that produces lesions in a variety of tissues including the skin, mucous membranes, connective tissues, and the eye. Reinoculation with CFA after subsidence of the disease results in an accelerated return. There is a genetic predisposition to the disease, as indicated by the variation in susceptibility among litter mates. Systemic injection of OT into diseased animals causes a transient increase in the severity of the joint involvement. Repeated injections of mycobacteria or mycobacterial fractions (without mineral oil) subcutaneously or i.p. (or very early in life, in the footpad) results in a loss of ability to develop the disease. Passive transfer of cells from sensitized donor rats (along with tuberculin) results in production of mild but typical lesions (1214). As would be expected, nocardias (including *Corynebacterium rubrum*) in oil induced the disease. Washed thoracic duct cells from diseased rats could transfer skin reactivity to PPD but not the disease. However, unwashed thoracic duct cells did transfer the disease. It has been concluded that adjuvant disease results from prolonged DH reactions to mycobacterial components deposited at sites of inflammation (915). Wax D in oil also produces adjuvant disease (951). Jollés et al. and Migliore (152) have confirmed the finding of Tanaka (1138) that acetylation of wax D eliminates its arthritogenic properties and have made the further observation that animals primed with acetylated wax D in oil are protected against subsequent challenge against nonacetylated wax D in oil. Cozine et al. (267) have used an adjuvant disease model in Holtzman rats in which the inducing dose is given at 28 days. They have found that pretreatment of the animals with *Mycobacterium smegmatis* (*butyricum*) in mineral oil (either 12.5- or 50-μg doses) protected them against induction of adjuvant disease 28 days later. Pretreatment with mineral oil alone afforded moderate but significant protection. Pretreatment with mineral oil failed to protect against induction at 14 days. There was moderate protection by 50 μg of the complete adjuvant for the 14-day challenge. It is indeed provocative to find mineral oil alone suppressing induction of arthritogenesis. The authors have suggested that if mineral oil caused the production of suppressor T lymphocytes, these might inhibit induction of the disease.

Adjuvant arthritogenesis provides a measure of potential side effects of mycobacterial vaccines when they consist of mycobacterial products in oil (see section on BCG and Mycobacterial Vaccines).

The ultimate mycobacterial adjuvant. As early as 1964 Jollès et al. looked into the basic requirements for adjuvant activity in the mycobacterial cell wall fragment, then known as wax D. They concluded that the amino sugars in the peptidoglycolipid were important for adjuvant action (557). Ishibashi and his associates have shown that wax D, alone, enhances the

immune response to ovalbumin (539). Azuma et al. (70) indicated that the walls of *M. smegmatis, M. phlei, M. tuberculosis* Aoyama B, *M. bovis* BCG, and *M. kansasii* were all potent adjuvants. Kotani has also long been interested in determining the location of the immunopotent configuration(s) responsible for adjuvant action in the CMN group. Japanese and French workers concerned with this matter have made remarkable progress over the last 5 years. In focusing on the nature of adjuvant-active material, two approaches have been used.

From Lederer's laboratory (6) has come a report of a water-soluble adjuvant (WSA) derived from purified mycobacterial cell walls (pretreated with trypsin and pepsin) by treatment with lysozyme. WSA consists of *at least* cell wall oligomeres of Ala-Glu-*meso*-DAP-GlucNAc-MurNAc (Fig. 19) of average molecular weight 20,000. WSA, substituted in the place of mycobacteria in CFA (WSA-FA), exhibited a stronger immunopotentiating effect than an equal amount of mycobacteria. Chedid and associates have shown that WSA-FA does not elicit hyper-reactivity to endotoxins, lymphoid hyperplasia in immunized animals, or polyarthritis in rats. Further, they have found that it greatly enhances the production of antiviral antibodies against Columbia SK virus (6). Subsequently, from this laboratory has come the description of a neo-WSA (from *M. smegmatis* and *N. opaca*) that enhances antibody response to ovalbumin (in guinea pigs) but does not produce DH to tuberculin or other "side effects" such as an increased susceptibility to histamine, hyper-reactivity to endotoxin, or hypertrophy of spleen and liver (7). Thus, WSA-FA lacks the side effects associated with CFA (see also [1098]).

Modolell et al. have recently shown that WSA-FA acts in vitro to increase the immune response of mouse lymphoid cells against SRBC, dinitrophenyl (DNP)-dextran, and DNP-edestin (hemp seed globulin). The primary action of WSA-FA seemed to be upon macrophages rather than a direct B cell-stimulating effect. There was no evidence for its adjuvant action being T cell mediated. Fetal calf serum gave much less effective results as a supplement for the antibody response in cell culture than did mouse PE fluid (841). Lederer and associates have reported that WSA contains: 12 to 15% amino sugars (GlcNAc and MurNAc in equal proportions); 60 to 70% arabinogalactan (with arabinose and galactose in a 2:1 ratio); 12 to 15% amino acids (principally Ala, Glu, and α,ϵ-DAP in a molar ratio of 1.3:1:1 and traces of other amino acids); less than 5% FAs; and no evidence for contaminating lysozyme. The authors further *suggest* that no mycolic acids are present (6).

This general approach to the production of WSA-FA from delipidated organisms has been extended (248) to *Nocardia corallina, N. rubra,* and *N. opaca,* with interesting results. For example, all fractions enhanced antibody production. All fractions were mitogenic except one lacking murein. Since that one did enhance antibody production, the enhancement must have been stimulated by a component other than murein, or undetectable traces of murein must have been present.

From Osaka have come two approaches to localizing adjuvant activity of cell wall skeletons and fractions of mycobacteria. (i) Azuma and associates (69) have examined the adjuvant behavior of cell wall skeletons (CWS; mycolic acid-arabinogalactan-murein) from BCG, *Nocardia asteroides* 131, and *C. diphtheriae* PW8, each *suspended in IFA*. Each was capable of enhancing the formation of circulating antibody and DH to BSA, SRBC, and sulfanylazo-BSA. More extensive studies were carried out with the BCG-derived CWS, which, in addition to the already mentioned enhancement of antibody formation both to carrier and hapten (BSA versus sulfanylazo-BSA), stimulated T cell-mediated cytotoxicity when administered along with target cell antigen. Neither arabinosemycolate nor arabinogalactan from BCG-CWS showed immunopotentiation. A significant side observation in these experiments was that oil droplets themselves, in combination with a dose of 10^5 mastocytoma P185-X2 cells, enhance cell-mediated cytotoxicity. On the other hand, CWS in buffered saline along with mastocytoma P185-X2 antigen (cells) did stimulate lymphocytes (specific "T effector cells") capable of causing the cytolysis of allogenic mastocytoma cells. Thus, although water-in-oil emulsion seems required for adjuvant enhancement of HI and DH, either oil droplets or CWS in buffered saline serve to enhance the development of specific lymphocytotoxicity. (ii) Kotani and associates (654) have used various enzymes for liberating and solubilizing moieties of the cell wall peptidoglycan from *M. smegmatis, C. diphtheriae* PW8, *Actinomyces viscosus, Bacillus megaterium, Lactobacillus plantarum, Streptococcus pyogenes* (group A, type 6), *S. salivarius, S. mutans, S. faecalis, Staphylococcus aureus, S. epidermidis,* and *Micrococcus lysodeikticus*. With the exception of material derived from the last two named species, preparations from each of the mureinic derivatives, administered in a water-in-oil emulsion,

showed a capacity to enhance the antibody response to ovalbumin as well as the development of DH. The authors had thought that the failure of mureinic fragments from *M. lysodeikticus* and *S. epidermidis* to behave as adjuvant might have been related to their lysozyme susceptibility, with consequent degradation in the host animal. However, as they point out, the strain of *S. epidermidis* used was lysozyme resistant. It is possible that these two strains may contain, in their mureinic fragments, one or more immunosuppressive components that counter any adjuvant effect.

The direction of all of this research has been to delineate that portion (or one of those portions) of the mycobacterial wall that is (are) essential for adjuvant action. Adam et al. (5) have found that the mycobacterial cell wall unit, *N*-acetylglucosaminyl-*N*-glycolylmuramyl-L-alanyl-D-isoglutaminyl-*meso*-diaminopimelyl-D-alanine, prepared by partial enzymatic degradation, gel filtration, and chromatography, when administered along with ovalbumin in IFA to Hartley female guinea pigs produced both an enhanced antibody response and DH. Material from *E. coli* cell walls similarly prepared, e.g., a mixture of disaccharide tetra- and tripeptides in IFA, elicited similar responses in guinea pigs. Further studies (342) with shorter fragments indicated that the *N*-acetylglucosamine and the carboxy-terminal D-Ala could be eliminated without loss of adjuvant activity. MurNAc tripeptides and a synthetic MurNAc dipeptide obtained from "Sinay et al." (811) were also active (see also reference 656).

Kotani and associates, in collaboration with a group associated with Shiba (655), have prepared several *N*-acetylmuramyl peptides (and *N*-acetylmuramyl-linked amino acids) by condensation of benzyl *N*-acetyl-4,6-*O*-benzylidene-α-muramide with various benzyl esters of peptide (or amino acid) employing the dicyclohexylcarbodiimide-*N*-hydroxysuccinimide or the ethylchlorocarbonate-*N*-methylmorpholine method, with subsequent removal of protecting groups by hydrogenolysis. *N*-acetylmuramyl-L-alanyl-D-isoglutamine was found to be the smallest unit showing the adjuvant activity ascribed to bacterial cell walls. In guinea pigs, the minimal amount of this *N*-acetylmuramyl dipeptide capable of enhancing synthesis of antibodies specific for, and DH to, crystalline egg albumin was 12.5 to 25 μg in a water-in-oil emulsion.

Up to the present, it has seemed that water-in-oil emulsion is a necessary adjunct for adjuvant action of any of a number of bacterial fractions. Recently, however, Audibert et al. have shown that there are exceptions to this rule (65). They have found that when antigen (BSA) is given *in saline* with MurNAc-L-Ala-D-iso-Gln or MurNAc-L-Ala-D-Glu there is a marked enhancement of HI. However, they offer no evidence that DH develops under these conditions. Furthermore, even with IFA and ovalbumin, MurNAc-L-Ala-D-Glu induces neither HI nor DH. Under similar conditions, MurNAc-L-Ala-D-iso-Gln induces both HI and DH. Thus, the γ-glutamyl function seems very important in the adjuvant action of the MurNAc dipeptide and especially so when IFA is used. When MurNAc-L-Ala-D-iso-Gln (5 μg) and MurNAc-L-Ala-D-Glu (5 μg) were used along with the controls, PPD (50 IU), OT (50 IU), and WSA (5 μg), they failed to elicit DH in animals sensitized with CFA. Animals given IFA plus MurNAc-L-Ala-D-iso-Gln (50 μg) and IFA plus MurNAc-L-Ala-D-Glu (50 μg) failed to become sensitized to any of the three elicitins.

Mycobacterial Vaccines

Living BCG. Freund's early confirmation of the fact that living *M. tuberculosis* exhibits the adjuvant properties of dead *M. tuberculosis* in CFA indicated that a living attenuated strain of *M. tuberculosis*, as a vaccine, might have broad immunogenic effects. The bile-tolerant, attenuated strain of *M. bovis* isolated 68 years ago by Calmette and Guérin (213) meets just these requirements. A number of BCG vaccination programs have been carried out in human populations in various parts of the world, and careful records of them point up the virtues and the shortcomings of BCG. BCG-induced tuberculin positivity persists for several years as shown by Comstock et al. (256) and is comparable to hypersensitivity induced by *M. tuberculosis*. Sutherland (1112) has pointed out that the actual protection afforded by BCG has varied from almost none to a remarkable 80%. This has puzzled a number of observers and has been attributed to possible variations in preparations of BCG vaccines (493) and to the regional occurrence of low-grade infections caused by other mycobacteria, such as *M. intracellulare*, with consequent cross-protection not significantly modified by BCG (904). Sutherland has looked at the relation between the incidence of tuberculosis in unvaccinated controls of particular regional BCG trials and the percentage of protection from BCG. Table 8 is adapted from his compilations. Sutherland's arrangement of trial data indicates the likelihood that benefits from BCG will be most evident in groups at special risk of exposure to *M. tuberculosis*. As an outstanding example, he cites the U.S.

troops sent to Vietnam (1113). The risk of tuberculosis there was high, and the infections developing in a group of Marines was as might have been anticipated, 5.3%, in an unvaccinated group being moved into a high-risk area (341). Furthermore, conversions to tuberculin positivity was, in a larger group of Americans in Vietnam, 4.9%. Of these, caucasians had a 3.4% conversion rate, whereas 17.1% of negroes converted (1077). For relevant studies on risk of tuberculosis disease in children in France, Poland, Switzerland, and Yugoslavia during the years 1961 to 1966, see reference (736).

It is a general truth that infection with most mycobacteria, including *M. tuberculosis*, can lead to tuberculin positivity (333, 1063). In fact, it has been found that school children in Queensland generally showed greater reactions to PPD-A (from *M. avium*) than to PPD-S. Even after vaccination with BCG, this is the case. However, a group of children who had been vaccinated with BCG shortly after birth and tested with PPD-A and PPD-S showed a preponderance of greater reactions with PPD-S (for at least 16 years after vaccination). Abrahams has called this "original mycobacterial sin" and has suggested that the first mycobacterial infection may set the "antigenic reaction pattern" (2). Thus, superinfection of BCG-vaccinated individuals should enhance the DH of these subjects, much as repeated tuberculin testing may prevent the waning of BCG-engendered immunity (897). Sutherland appears to be among the first to question the importance of superinfection (mycobacterial infections encountered after BCG vaccination) upon the duration of BCG-induced immunity.

TABLE 8. *Efficacy*[a, b] *of BCG vaccination as related to the incidence of tuberculosis*

Trial vaccination	Tuberculosis per 1,000/yr among unvaccinated	%[b] protection from BCG
North American Indians	15.6	80
Chicago infants	2.2	75
British schoolchildren	1.3	78
South Indians, rural	0.86	60
Puerto Rican children	0.43	31
Georgia, Alabama	0.13	14
Georgia, schoolchildren	0.11	nil

[a] After Sutherland (1112).

[b] "By relating the percentage efficacy to the incidence of tuberculosis in the unvaccinated group *alone*, any relationship between efficacy and the amount of tuberculosis will be obscured to a greater or lesser extent by a spurious correlation. This is the intrinsic correlation between any compound variable (in this instance the ratio of the rates in the vaccinated and unvaccinated groups) and one of its parts (the rate in the unvaccinated group). In the present instance a chance fluctuation which increases the unvaccinated rate above its true value will reduce the ratio of the vaccinated to the unvaccinated rate, and vice versa; the effect of the chance variability of the unvaccinated rate is consequently to create a correlation which will be superimposed on, and which will therefore obscure, a genuine relationship between the efficacy of vaccination and the incidence of tuberculosis in the area. . . . The misleading effects of this correlation can be avoided by relating the percentage efficacy instead to an *average* (namely the geometric mean) of the incidences in the vaccinated and unvaccinated groups. When this is done the close concordance between efficacy and the incidence of tuberculosis in the seven controlled trials remains. Regarding the two trials in Georgia as one, the chance of as close a concordance is still only 1 in 60" (1113).

The standards for BCG dried vaccine were issued by the World Health Organization 10 years ago (1283). The most common danger that could introduce variability in trials is that of not using the vaccine soon enough after hydration, with consequent loss in viability, e.g., following storage at room temperature. When a BCG strain has been maintained by serial subculture for several years, changes in genetic properties have been observed (509). Thus, maintenance of all stocks in the lyophilized state is indicated. When vaccines from two separate countries, strains Copenhagen 1331 and Glaxo 1077, were compared in a trial with school children, they were found to be equally effective (186; see also [232]). In other cases, differences *have* developed in vaccine strains. For example, differences have been found (i) in sensitizing capacity of various strains (946) and (ii) in the overall lipid content of compared vaccine strains of BCG and strains of *M. microti* (1108). These changes were associated with certain periods when stocks underwent lyophilization.

Asselineau and Portelance (64) have made a comparative study of the free lipids of eight sublines of BCG: Danish COP 3, Japanese, Montreal I.M.H., Moreau (Brazil), Paris I.P., Russian, Swedish D-3, and Tice 946 BL. The total free lipids ranged from 21 to 31% of the dry weight of the bacterial cells. Fractions from ethanol-ether extracts were: fats, with a range of from 9 to 18%; phospholipids, 4.3 to 6.7; wax A, 3 to 11.5. Fractions from chloroform extracts were: wax B, range 0.1 to 0.4%; wax C, 0.04 to 2.7; wax D, 0.02 to 0.2. Free FAs (extracted in an aqueous solution of Na_2CO_3) varied from 8.5

to 49.2% of the total fat fraction. The authors have stressed the importance of differences found in amounts of cord factor and amounts of wax D in the different strains. They have indicated that both the Montreal and Moreau strains give good results in tuberculosis vaccination, whereas the Tice strain is less effective. The former two strains contained three times as much cord factor as the latter. However, the wax D content of the three strains was equivalent. These data were presented at a symposium on cancer therapy, and the authors conclude with the following statement: "the production by a particular BCG daughter strain of large amounts of cord factor and of waxes D rich in peptidoglycolipid might be a factor to consider in the choice of a strain for tumor immunotherapy." It should be noted that the implied modifications in the BCG strains have occurred over a long period of time, involving many transfers on a variety of media. It is to be hoped that the chemical expertise of such investigators as those in the Toulouse group will soon be directed towards the study of specific, genetically related, mutants selected for their manifestation of mutant lipid patterns. At the onset, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced mutants could be selected on the basis of colonial morphology. In the case of mycobacteria, there is good reason to expect the appearance of such mutant colonies to be related to changes in products of lipid biosynthesis.

A regional lymphadenitis (BCG-itis) has been found in some trials to be a side effect in as many as 1.9% of vaccinated children. A controlled study of such a condition in Panama suggested that it was associated with the use of vaccine initially prepared as a fluid suspension and that, in 2,000 children inoculated from freeze-dried preparations, BCG-itis did not occur (241).

Some years ago Horwitz and Meyer prepared a scholarly treatise on the safety record of BCG vaccination. They set the normal reaction to the intracutaneous injection of BCG:

> During the first 14 days after the vaccination no local reaction appears at the site of injection. Following this period an infiltration slowly develops which later ulcerates. It reaches its maximum from 3½ to 6 weeks after vaccination and should not measure more than 11 mm diameter at this time. The ulceration should heal no later than 4 months after the vaccination. The regional lymph glands should not be suppurating or become adherent to the skin. Other clinical symptoms should not appear. *This clinical course must be considered the response of the normal, non-allergic* [established by prior tuberculin testing] *organism to intracutaneous injection of BCG in the doses generally used.* If the reaction is appreciably stronger, and/or other clinical symptoms appear, the course of the BCG vaccination must be considered complicated (521).

In places where tuberculin testing prior to BCG vaccination is an added burden, it has been reported (from Mongolia) that vaccination without prior tuberculin testing does not cause untoward reactions even in tuberculin-positive individuals (242).

The adjuvant effects of BCG upon an immune system already challenged with "cancer" have been the subject of many papers prior to, and during this, the seventh decade of BCG. The possibilities and limitations of BCG for immunoprophylaxis and immunotherapy of cancer have recently been reviewed by Bast et al. (101).

Living BCG or BCG cell walls in oil when given in conjunction with, for example, the ascites form of line 10 of the hepatocellular carcinoma of guinea pigs, induce tumor rejection immunity (100). Whether or not there are antigens shared by BCG and the carcinoma cells is not known. Shared antigens have been demonstrated between human malignant cells and BCG (822). *B. pertussis*, when given along with BCG and the mouse ascites tumor cells, suppresses the immune response (100). In another system employing killed BCG or delipidated and deproteinized $H_{37}Ra$ and tumors in guinea pigs, tumor regression could be much enhanced by the addition of cord factor to the bacterial vaccine (118). Thus, whereas the T cell-oriented adjuvant *B. pertussis* suppresses the development of tumor rejection, trehalose dimycolate enhances rejection.

It is clear from the discussion in Ultimate Mycobacterial Adjuvant that a variety of fragments of mycobacterial cell walls will have the capacity to enhance three aspects of the immune response: HI, DH, and lymphocyte cytotoxicity (see also Cord Factor Inhibition of Tumors). While it is beyond the scope of this review to cover all of the papers on this general topic, the interested reader should see papers from the group working with Ribi (e.g., references 72, 463). This group has carried out a number of studies concerning the adjuvanticity of a fraction, termed P3 (a mixture of trehalose mycolates [463, 705]) from *M. tuberculosis*. P3 plus PPD served to stimulate DH to PPD.

Residues of methanol-extracted BCG (MER). Weiss and various associates have found that the residue from methanol-extracted

cells of BCG (MER), when administered in IFA (MER-FA), markedly enhanced the immune response to such poor antigens as DNP conjugates of guinea pig globulin and human serum albumin. When antigen and MER-FA were given jointly, both HI responses (measured as hemagglutinin) and DH responses (skin test positivity) were elicited. MER-saline did not serve as an adjuvant. However, MER-saline, unlike MER-FA, could be used in pretreatments (no antigen) which, depending on (i) time of pretreatment, (ii) subsequent immunizing antigen, and (iii) amount of that antigen, could somewhat modulate the immune response to DH or HI (121). MER (0.5 to 1.5 mg) is reported to have markedly curtailed a naturally occurring epidemic of *Pasteurella pseudotuberculosis* among a large population of guinea pigs, a fraction of which just happened to have received MER pretreatment. MER in the correct doses also has been shown to be beneficial in tumor rejection. Weiss prefers to categorize MER as an immunological modulator rather than as an adjuvant. He notes that, although MER is a macromolecular lipopolysaccharide-protein complex, it behaves very differently from gram-negative endotoxin lipopolysaccharide: e.g., it is not pyrogenic and it lacks the toxic properties associated with the activation of complement and various kinins (1240).

***Mycobacterium microti* and the vole bacillus vaccine.** In 1937, Wells made the first report of tuberculosis in *Microtus agrestis*, the vole. *Mycobacterium microti* proved to be deadly for guinea pigs and produced large local lesions in rabbits. One month after inoculation, both guinea pigs and rabbits became tuberculin positive (1246). Wells became interested in developing a vaccine from an attenuated strain of *M. microti* to be used in humans for protection against tuberculosis. He has described the development of his vaccine strain. The following are his deliberations about this strain and the apparently fully attenuated strain of *M. microti* employed by Šula and a Czechoslovakian group (1107):

> The fact that the vole bacillus is a natural pathogen of voles and its virulence for voles can be maintained by passage through its natural host is a considerable theoretical advantage over BCG, which is a bovine type of tubercle bacillus attenuated to the point where it is no longer pathogenic to laboratory animals. The virulence of the vole bacillus for voles diminishes, as does that of the great majority of pathogenic mycobacteria, with repeated subculture on egg media. By passage through laboratory-bred voles after each tenth subculture on egg medium the vaccine strain of vole bacillus has, however, retained its full virulence for voles after nineteen years. The same strain, but not passaged through voles, is now almost avirulent for its natural host.
>
> This is the appropriate place to consider why the strain of vole bacillus used by the Czech workers has given so low a complication rate and so small a local reaction compared with the vole bacillus vaccine used in Great Britain, while still conferring a high degree of tuberculin hypersensitivity on the vaccinated. It appears from the literature that *Šula's* strain has not been passaged through voles for nine years. It was subcultured in a synthetic liquid medium to which ascitic fluid was added, and later in the synthetic medium without ascitic fluid. During this time it is clear that considerable attenuation must have taken place, as the dose injected intradermally in man is 0.05 mg. bacilli "semi-dry weight". Newborn babies receive twice this dosage. *Wells* and *Wylie* (1249) have found that a dose of 0.0001 mg. moist weight produces a local reaction in man comparable with the usual dosage of the Copenhagen strain of BCG. *Gaisford* (413) has also reported that a dose of vole bacilli far lower than the dose of BCG used by him in newborn babies produces a brisk reaction with an appreciable complication rate. The question, therefore, arises whether a degree of attenuation in the vole bacillus for the preparation of a vaccine for use in man is desirable. It certainly seems that the vaccine used by the Czech workers has most desirable qualities. It produces a smaller local reaction than BCG, the complication rate is lower and the tuberculin hypersensitivity is greater. But how is this level of virulence to be maintained? The answer to this question is not clear (1247).

From a trial carried out in 1950 with certain batches of vole bacillus vaccine, untoward reactions developed which became obvious 2 to 3 years or more after vaccination and which persisted as a skin condition termed *lupus murinus* (188, 761). Over a 15-year period, some of these lesions had grown to cover a wide area of the upper arm. Bacilli isolated from these lesions were incapable of initiating infections in 12 field voles (*Microtus agrestis*). They were inoculated directly with biopsy material from patients (761). As early as 1957, Hart et al. gave an assessment of tuberculosis vaccines used in adolescents in Great Britain and concluded that "although both BCG and vole bacillus vaccines have so far produced a similar degree of protection, lupus has been observed to develop at the site of vaccination in some of the participants who were given vole bacillus vaccine, but not following BCG" (497). Wells died in 1956 (1274). Some of the difficulties with Wells' strains were publicized in the *British Medical Journal* under an anonymous "leading

article": "Of Voles and Men" (188). Since the fully attenuated strains used in Czechoslovakia have been a success, the lupus problem with only three of Wells' substrains would seem to offer no insurmountable hurdles in perfecting Wells' living vaccine from *Mycobacterium microti*. In fact, just such a recommendation has been made in an editorial appearing recently in *The Lancet* (686).

Cross-protection studies with mycobacterial vaccines. Collins (255) has shown that pathogen-free CD-1 mice vaccinated intravenously with 10^6 living *M. tuberculosis* Erdman; *M. bovis* BCG-Tice; *M. avium* serotype 1; *M. kansasii* strain Forbes or Brownell; or *M. intracellulare* D673 (serotype Chance) were satisfactorily protected against subsequent challenge with *M. tuberculosis* Erdman or with *M. bovis* BCG. However, mice immunized with *M. bovis* BCG SM^R; *M. kansasii* Bostrum; *M. scrofulaceum*; *M. intracellulare* strains Trudeau 1403 (serotype Boone), 1406 (serotype Yandle), and 1467 (serotype Altman); *M. terrae* or *M. fortuitum* were not protected. The property common to these latter ineffective strains was an inability to survive in the mouse. This property operationally served to render them both nonimmunogenic and nonallergenic (in the mouse). The finding of the ability to survive in the mouse in only one of four strains of *M. intracellulare* emphasizes the importance of examining representative strains in reaching conclusions about virulence (as well as any other property) of mycobacteria. Collins has attributed the non-immunogenicity of these strains to their inability to survive in *macrophages*. This would seem to be really an inability to form in vivo phenotypes. (Collins has also dealt with the question of the interference of one mycobacterial infection [primary] with a second infection [vaccination]. His conclusions and those of Palmer and Long [904] indicate that a concurrent mycobacterial infection is not likely to interfere with the development of adequate levels of antituberculous resistance in response to vaccination with BCG [255].)

Cell wall vaccines. In a number of papers, Ribi and associates have tested the protective efficacy of vaccines prepared from oil-treated mycobacterial cell walls (970), employing Rhesus monkeys and airborne infection. In a study of cross-protection between BCG and $H_{37}Rv$ in mice, Ribi et al. have emphasized the specificity of immunity demonstrated by the aerosol challenge as compared with i.v. challenge (971). This finding may have important implications concerning the assessment of the mouse-protection test (employing i.v. challenge) in relation to immunity to infections which, in nature, are airborne.

Alsaadi and Smith have shown that in guinea pigs infected by the respiratory route and followed for 18 weeks: (i) $H_{37}Rv$ rapidly increased 100,000-fold and maintained this level in the lungs for the period of observation; (ii) $H_{37}Ra$ increased 1,000-fold up to week 3 and slowly underwent an elimination from the lungs, completed by week 12 (both $H_{37}Rv$ and $H_{37}Ra$ were recovered from lymph nodes and spleens); (iii) in contrast, *M. smegmatis* failed to multiply in the lungs of guinea pigs following inhalation of 2,000 viable bacilli (27). Thus, route of exposure is of paramount importance in infections, and the natural route is the only one that can make sense relative to contraction of disease under natural conditions.

MYCOBACTERIOPHAGES

Redmond has written a review of mycobacteriophage studies up to 1963 (962). In Fig. 29, 30, and 31 are illustrated several bacteriophages active on mycobacteria. The scant information available indicates that these are all double-stranded DNA phages (809) with a G+C content close to that of mycobacteria (660). Most of the mycobacteriophages illustrated in this paper belong to class B of Bradley (174), the exception being I3. Bradley included no mycobacteriophages in his landmark review. Group B phages are characterized by a hexagonal head and a long noncontractile tail. Kölbel and Mohelska have presented an informative study of four of these phages of group B (AG1, GS4E, D29 and BK1) and suggested that the tail base is a pentahedral structure with fibrous appendages (630). In Fig. 32 is illustrated a composite analysis of a number of group B mycobacteriophages that we have studied. Evidence to support the tail structures we have inferred can be found by careful examination of Fig. 29 and 30. Additional information concerning tail structures is given in the legends to Fig. 31 and 32. Phage I3, a mycobacteriophage of group A, is remarkable as a bacterial virus active on members of the CMN group, since it has a tail structure that includes a baseplate, collar, and a contractile tail such (except for its lack of tail fibers) as are found in the T-even coliphages (10, 658).

A diagram of the overall structure of mycobacteriophage MC3 is shown in Fig. 32. Its distinctive features include a hexagonal head (63 by 70 nm) and a tail which, at its middle, is about 11 nm across and which tapers to 8 nm at the head end and 8 nm at the end bearing the basal structure and tip. The basal structure has

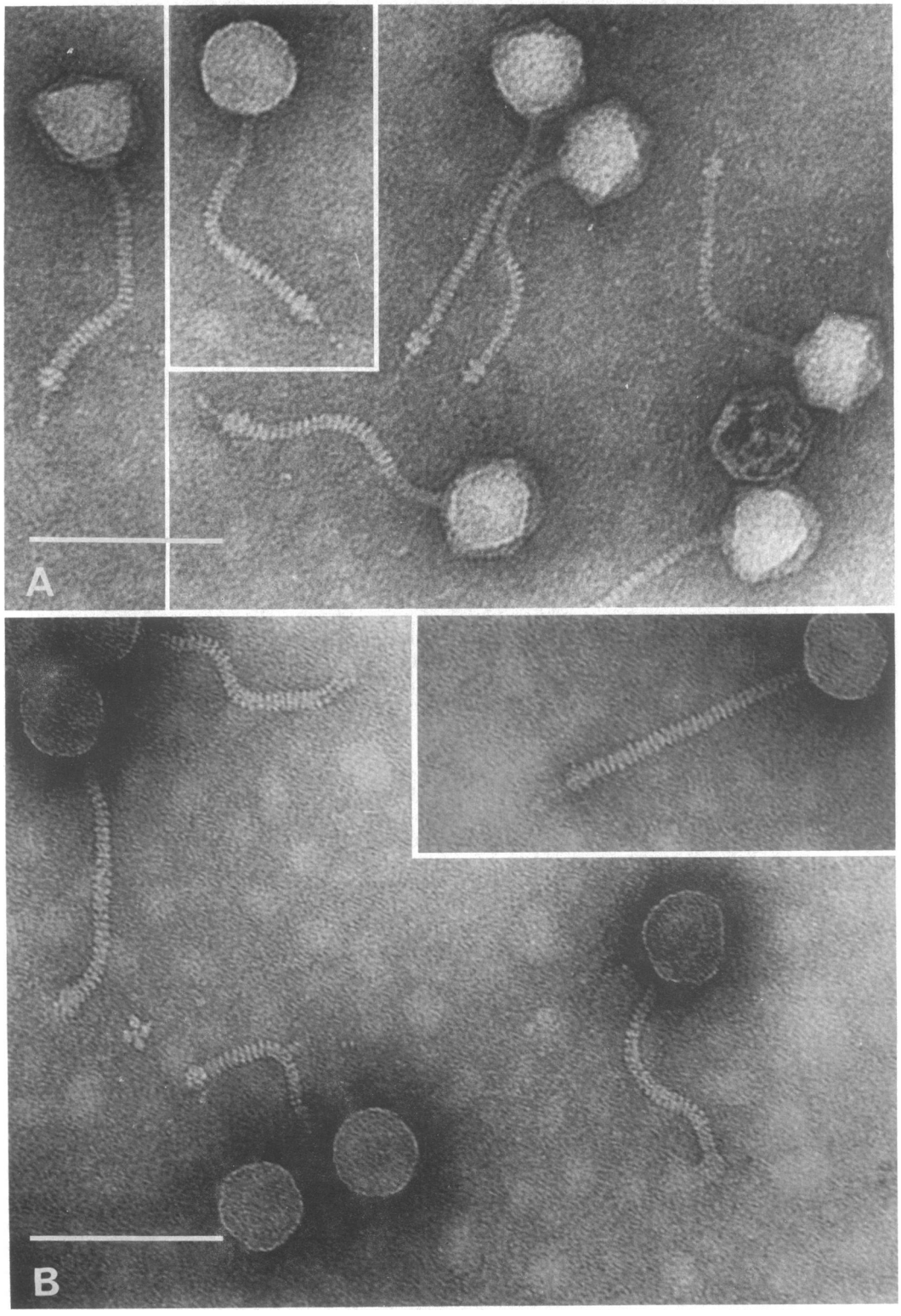


FIG. 29. *(A) Mycobacteriophage MC-1. Somewhat smaller in dimensions but strikingly similar in structure to MC-3. Note the distinct cross-striations and the single fiber extending from the tail plates. (×300,000; bar = 0.1 μm.) (B) Mycobacteriophage MC-4. Hexagonal head structure is not clearly demonstrated. Note distinct cross-striations, three to four ringlike baseplates, and fibers. (×300,000; bar = 0.1 μm.)*

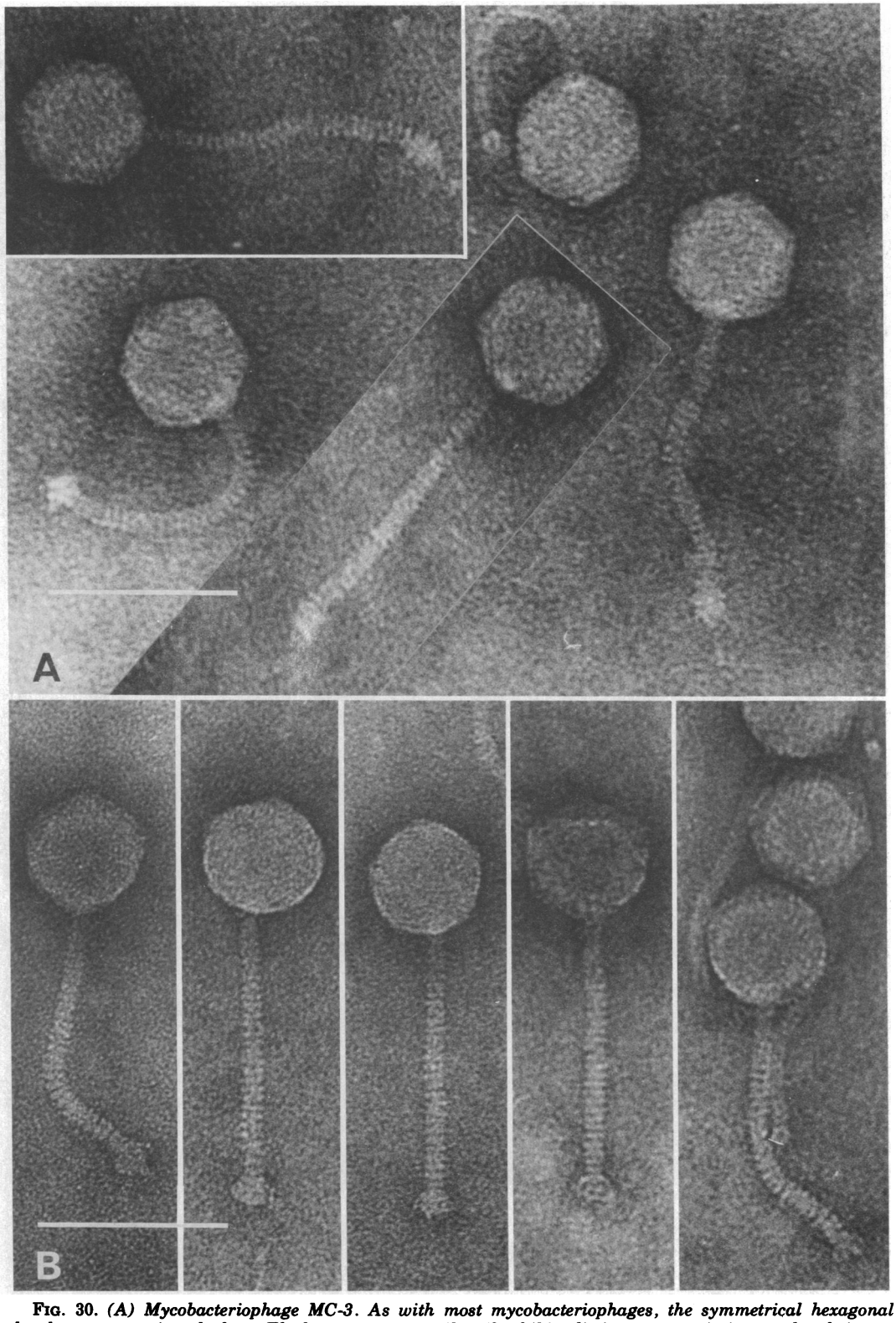
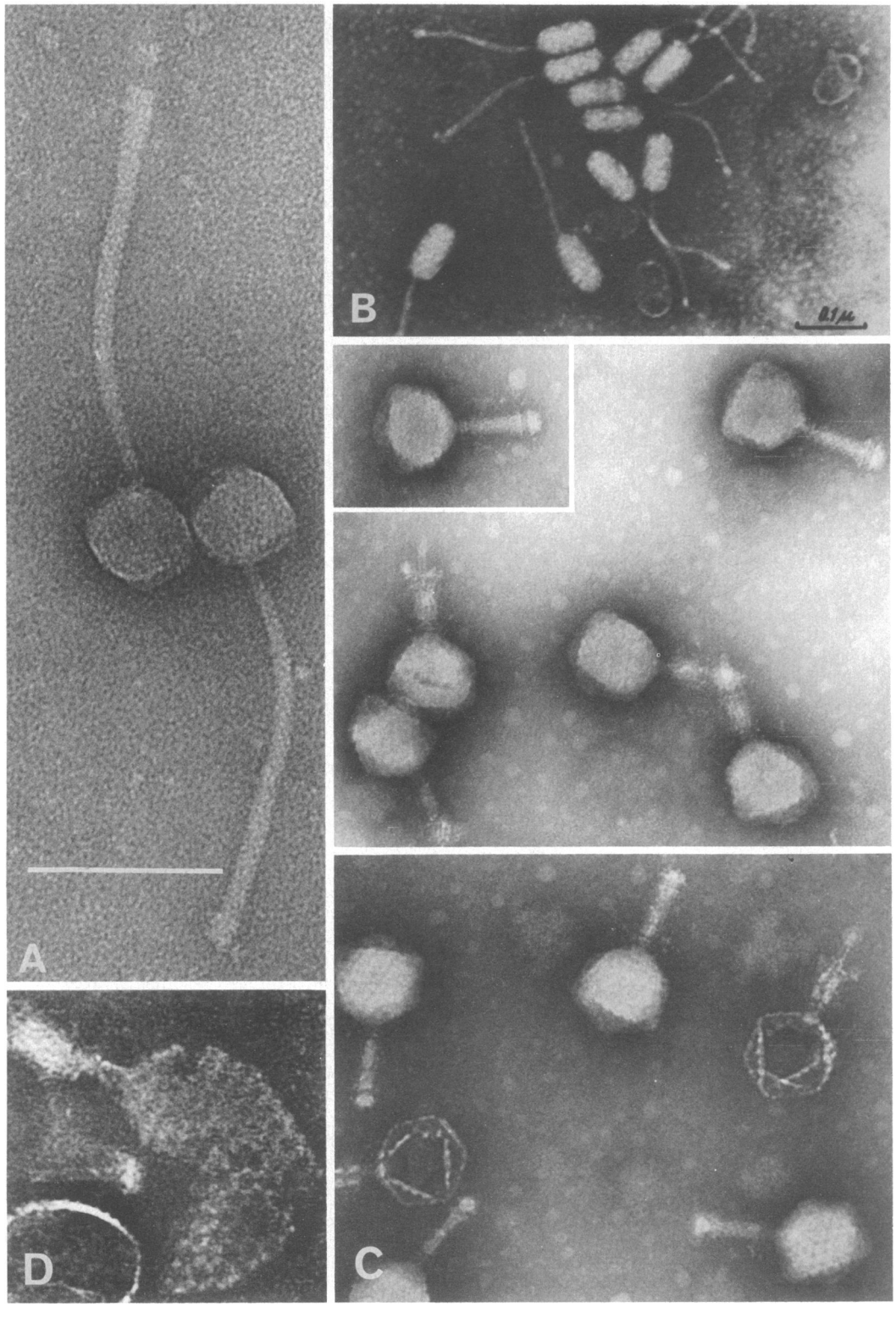


FIG. 30. *(A) Mycobacteriophage MC-3. As with most mycobacteriophages, the symmetrical hexagonal head suggests an icosahedron. The long, noncontractile tail exhibits distinct cross-striations and ends in one or more fibers extended from two parts of the baseplate. (×300,000; bar = 0.1 μm.) (B) Mycobacteriophage D29 exhibits geometrical structures similar to those of MC-3. Its tail has distinctive baseplates suggestive of an umbrella or lamp shade. In some the spikes appear with no interconnecting webbing. no tail fiber has been observed. (×300,000; bar = 0.1 μm.)*


A
B
0.1μ
C
D

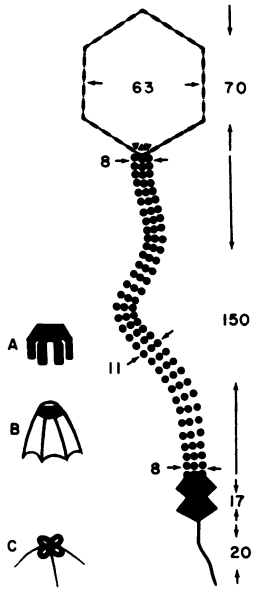


FIG. 32. *Schematic drawing of a typical mycobacteriophage (MC-3) revealed in ammonium molybdate-stained micrographs. Five capsomeres appear to be arranged on each face of the hexagonal sides. The long, flexible tail consists of about 34 units (cross-striations), two distinct baseplates, and a fiber. (A) and (B) are baseplate models of phage D29. (C) is that of phage MC-4. All dimensions are represented in nanometers.*

the appearance of an expansion valve with an apical fiber. Examination of the negatively stained electron micrographs of MC3 reveals 34 subunits in the tail and the constricted terminal swelling with its fiber. Along the edge of the faces of the head, one can count five capsomeres. Phage D29 (Fig. 30B) differs from MC3. Its tail structure sometimes suggests structure *A* in the diagram and sometimes structure *B*. Our diagram of the terminal apparatus of MC4 appears as *C* in Fig. 32. In Fig. 31A is shown phage C-1 of Mankiewicz (767), the hexagonal heads of which contrast with the oval heads of phage F-K of Buraczewska et al. (Fig. 31B). Table 9 offers a list of representative mycobacteriophages with their major structural features.

Serology

As with other viruses, the simplest technique for the serological characterization of mycobacteriophages is the neutralization of infectivity with specific antisera. Phages of the CMN group are said to be poor antigens (163, 685). However, there are published examples of phages that induced the formation of relatively good neutralizing sera (164, 838, 1045) and others where the titers of the antisera were low (166, 380, 660). Bowman has compared the capacity of sera prepared against phages R1, D29, and Leo to neutralize heterologous infectivity and found no cross-neutralization; i.e., the fixing and adsorption apparati of each of these phages seem serologically distinct (164). Castelnuovo et al. (223) have examined the protein antigens of phage *phlei* liberated by KOH hydrolysis, and Castelnuovo et al. (220) have studied, by immunodiffusion and immunoelectrophoresis, the antigens of whole and disrupted phage *phlei*. Using antiphage *phlei* sera they have looked for lines of identity with antigens from six other mycobacteriophages. Two shared lines were found between phage *minetti* and phage *phlei*, but no sharing was apparent with antigens from the other five phages, which included D12, D29, and D35. Since phage *minetti* came into being (by recombination between resident prophage and phage *phlei*?) following the growth of phage *phlei* in the *minetti* strain of *M. fortuitum*, some antigenic sharing between the two might be expected. What is impressive is the lack of serological relatedness between six of the seven phages. Thus, the experience of several investigators indicates that mycobacteriologists have at their disposal a number of serologically identifiable and distinct phages.

Lipoidal antigens of mycobacteriophages have not yet been reported (166, 1046). As will be seen from the next section, lipoidal components are essential for the infectivity of some mycobacteriophages. That these lipids functions as antigens remains to be established.

Phages *phlei* have been employed in studies of the in vitro synthesis of antibodies by rabbit spleen cells (339, 510).

FIG. 31. *(A) Mycobacteriophage C1. Hexagonal head structure is not clearly shown. Cross-striations and baseplates are noticeably missing. The diameter of the tail at its juncture with the head is 7 nm. Its diameter at the end is 13 nm. This phage offers an exaggeration of the tail entasis typical of mycobacteriophages. (×300,000; bar = 0.1 μm.) (B) Mycobacteriophage F-K. (From reference 200, by permission of the author and publisher.) Shows an oval head and long, noncontractile tail with bulbous baseplate. (C) Mycobacteriophage I3. (From reference 658, by permission of the author and publisher.) This is the only mycobacteriophage to date which belongs to Bradley's group A. Note the large hexagonal head, the contractile sheath, and the hollow canaliculus spent of its DNA. (×180,000.) (D) Mycobacteriophage I3 (our preparation), showing disrupted phage head with closely packed globular capsomers (9.5 nm in diameter, and center-to-center distance is 13 nm) in hexagonal arrays. (×264,000.)*

TABLE 9. *Anatomy of representative mycobacteriophages*[a]

Mycobacteriophage	Dimensions (nm)		Terminal tail structure[c]	Tail fiber (number, nm)	Propagating *Mycobacterium*	Reference
	Head[b]	Tail				
MC-1	Icosahedron,[d] 50	125 × 10	2 Disks	1, 20	*M. smegmatis*, 607	K and B[e]
MC-3	Icosahedron,[d] 63	150 × 10	2 Disks	1, 20	*M. smegmatis*, 607	K and B
MC-4	Icosahedron,[d] 45	125 × 10	4 Rings	3-4, 20	*M. smegmatis*, 607	K and B
D28	Hexagonal, 65	170 ×[f]	Bulbous		*M. lacticola*, F13	1047
D29	Icosahedron,[d] 63	135 × 10	Plate with spikes		*M. smegmatis*, 607	K and B
D32	Hexagonal, 80	220 ×	Bulbous		*Mycobacterium* sp. F119	1047
D34	Hexagonal, 65	250 ×	Bulbous		*Mycobacterium* sp. F130	1047
C1	Icosahedron,[d] 50	200 × 13	Swollen club	N.O.[g]	*M. smegmatis*, 607	K and B
C2	Icosahedron, 60	200 × 7.5	Platelike		*M. smegmatis*, 607	810
AG1	Hexagonal, 65	120 × 10	Bulbous		*M. kansasii*	200
BG2	Icosahedron, 80	280 × 15	2 Disks	2, 70	*M. smegmatis*, 607	200
BK1	Octahedral, 80	280 × 16	Bulbous	2, 70	*M. smegmatis*, 607	200
GS7	Hexagonal, 83[h]	135 × 12[h]	Bulbous		*M. smegmatis*, 607	533
67	Hexagonal, 60	180 × 15	2 Disks	2, 70	*M. pellegrino*	200
MyF3P/59a	Hexagonal, 52	123 ×			*M. fortuitum* (*minetti*)	843
B1	Octahedral, 50	145 × 9	Plate with spikes		*Mycobacterium* sp. Jucho	1130
B2, B3, A2, A3, A4, A5	Hexagonal, 70	140-170 × 10	Bulbous		*Mycobacterium* sp., Jucho	1133
Y7, Y10	Octahedral, 80	260 × 15	Bulbous	Short fibers	*Mycobacterium* sp., Jucho	200
3111-D	Oval, 90 × 45	150 × 15	Bulbous	Short fibers	*M. smegmatis*, 607	200
3215-D	Oval, 90 × 45	190 × 14	Bulbous	Short fibers	*M. smegmatis*, 607	200
F-K	Oval, 95 × 50	215 × 16	Bulbous	N.O.	*M. smegmatis*, 607	200
F-S	Oval, 95 × 50	235 × 16	Bulbous	N.O.	*M. smegmatis*, 607	200
R1	Oval, 100 × 60	200 × 20	Bulbous	Short fibers	*M. butyricum*	168
I3	Hexagonal, 85	85 × 12	Platelike		*M. smegmatis*, 607 or SN2	K and B, 658

[a] See Tables 1, 2, and 3.
[b] Hexagonal and octahedral: refer to outline of head of phage.
[c] Tail type: among the phages listed here, only I3 exhibits a tail having a contractile sheath. It is the only one of these mycobacteriophages belonging to Bradley's group A (174). All others belong to Bradley's group B.
[d] Most likely structure is an icosahedron.
[e] K and B, Kim and Barksdale, unpublished data.
[f] Blank spaces indicate that information is not available.
[g] N.O., Not observed.
[h] Estimated from data in reference.

Chloroform-Sensitive Mycobacteriophages

Some mycobacteriophages are exceptionally sensitive to chloroform (165, 380, 1046); others are not (955). Exposure of phages D4, D29, D28, and D32 in broth to 30% (vol/vol) chloroform for 1 h at 37°C followed by removal of solvent and evaporation to dryness (1 h), resulted in a drop in titer of 7 logs (1046). *N*-butyl alcohol was almost as effective. Ethyl ether produced a more marked effect on D4 and D28 than on D32 and D29. It was effective in bringing about inactivation even when lowered to 10% (vol/vol). Methanol and benzene varied according to the phage being examined. These results suggested that the integrity of the adsorption and injection apparati of these phages involved neutral and phospholipids. Bowman et al. (167) have examined highly purified, chloroform-sensitive mycobacteriophage DS6A grown on $H_{37}Rv$ and found 11.7% of its dry weight to be lipid. Of the phage lipid, approximately 53.8% (6.2% of the dry weight) was phospholipid. A comparison by TLC of $H_{37}Rv$ lipids and the lipids of phage DS6A revealed similar, but far from identical, patterns. Jones et al. (564) have reported that phage D29 contains no phospholipid. No phospholipid has been reported in phage R1 (362, 380). Thus, of the chloroform-sensitive mycobacteriophages, R1 (362, 380), Leo, D29, DS6A (165), D4, D28, and D32 (1046), only DS6A is known to have an essential phospholipid component. The only other bacteriophage known to have a major phospholipid component is the marine, ether- and chloroform-sensitive, tail-less, spiked pseudomonad phage, PM2, in which 75% of the total lipid has been reported to be phospholipid (351). Subsequently, in a paper concerning the molecular ultrastructure of PM2, preliminary data were given which established the percent phospholipid of total lipid as greater than 90 (492). The

mycobaceriophages, then, should be interesting to classify, for in addition to the usual properties of overall size, shape, DNA content, host range, and serological peculiarities, they may possess an essential lipid component, either a neutral lipid or phospholipid, or both or neither. These are characteristics that can easily be screened for by exposure to lipid solvents followed by assay for viral activity.

Lysogeny and Pseudolysogeny

The greatest number of wild-type phages in nature exist in lysogenic bacteria (97). Among common examples of lysogenic mycobacteria are *M. tuberculosis* $H_{37}Rv$ (1111), *M. bovis* (531), *M. marinum* (1059, p. 54), *M. fortuitum* (883), *M. smegmatis* (155, 532), and others (201, 308, 1200). An impressive study concerning the lysogenization of mycobacteria was carried out by Russell et al. in 1960 (1003). They were able to establish lysogenic strains of *M. tuberculosis*, *M. smegmatis*, *M. phlei*, and *M. fortuitum*. Investigators of lysogeny in mycobacteria have often rediscovered the fact that the demonstration of lysogeny is much facilitated when there is available a suitably sensitive (indicator) bacterium. Many papers about *experimental* lysogeny in mycobacteria have in fact been about the phage carrier state or pseudolysogeny (97). Baess has reviewed pseudolysogeny and presented a case in point (80). Russell et al. (1003), in their studies, were well aware of the possible confusion of lysogeny with pseudolysogeny and went to considerable lengths to obviate such confusion. If corynebacteriophages can be taken as models of the CMN group, then it can be said that most lysogenic mycobacteria are resistant to phages homologous to their carried prophages (518, 742) and rare exceptions to this generalization (97) appear to be of little consequence outside the experimental genetics laboratory. This resistance, lysogenic immunity (742, 947), results from the synthesis of a specific prophage-encoded repressor which shuts off the expression of viral functions in the carried phage genome. Russell et al. (1004) were the first to do a controlled study of the effects of lysogenic immunity on the response of mycobacteria to typing phages. The use of lysogenic immunity in phage typing is discussed further on.

Suitability of Mycobacteriophages as "Typing" Phages

In 1973, there was issued the following statement by the WHO Cooperative Study on the phage typing of mycobacteria:

> Although phage-typing of some pathogenic microbes—e.g., *Escherichia coli* and the genera *Salmonella*, *Staphylococcus*, and *Streptomyces*—is in routine use, little progress has yet been made in the phage-typing of mycobacteria, chiefly because mycobacteriophages possess a polyvalence that prevents the reliable identification of individual species of mycobacterium. Furthermore, laboratories cannot compare their results because they employ different methods and culture media and also because the phages used cannot be accurately identified. It has been shown that internationally reproducible results can be obtained for *M. tuberculosis* by selecting suitable mycobacteriophages and sensitive culture media, and by using a standardized technique of phage-typing. Under these conditions, phage lysis may be utilized to classify strains of *M. tuberculosis* (1109).

Despite the negative tone of this report, written 20 years after Lwoff's review on lysogeny (742), 14 years after the publication of Adams' *Bacteriophages* (10), 9 years after the appearance of the first edition of Hayes' *The Genetics of Bacteria and Their Viruses* (501), 20 years after Anderson and Felix presented accumulated facts about the Vi-type determining phages of *S. typhi* (37), and 16 years after Anderson had spelled out the broad effects of bacteriophages in bacterial ecology (36), there is every reason to believe that a satisfactory phage-typing scheme can be worked out for the mycobacteria. Mycobacteriophages are essentially like other phages. For example, there exists a large number of mycobacteriophages capable of replicating in a variety of mycobacterial hosts (567, 1004).

In Fig. 29, 30, and 31 are shown representative mycobacteriophages that vary in size and shape and complexity of tail structure. So far as we know, the genomes of these phages are found to be double-stranded DNA (809). RNA-containing mycobacteriophages and mycobacteriophages characterized by single-stranded DNA remain to be discovered. The isolation and purification of the phages do not seem terribly difficult, since Pokorny and associates report ultrafiltrates of phage D29 (Fig. 30B) of 4.2×10^{15} particles per ml (932). The preparation of functional DNA from mycobacteriophages has been accomplished in successful transfection experiments by Tokunaga and Sellers (1158) and by Nakamura (867). Mycobacteriophages respond to the mutagenic effects of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (838). As already pointed out, mycobacteriophages range from good inducers of specific phage-neutralizing antibody to those behaving as moderately good to poor antigens.

It has long been known that certain mycobac-

terial host strains permit the replication of certain mycobacteriophages at one range of temperatures. For example, 6 of 9 different strains of *M. avium* resisted the destructive effects of six phages when growth occurred at 37°C. When growth occurred at 42°C, there was a differential pattern of susceptibility: some phages grew in some strains and not in others (406). The discoverers of this example of temperature-sensitive permissiveness in mycobacterial phage-host systems pointed out its potentialities for lending additional discriminatory powers to a set of typing phages. Phage geneticists use temperature-sensitive mutants (*ts* mutants) of phages as sources of markers for exploitation in genetic mapping. Mizuguchi and Sellers have used matings between *ts* mutants of mycobacteriophages D29 and D29A for establishing a rudimentary genetic map of phage D29 (838).

Not only can mycobacteriophages be readily mutagenized, any phage stock of 10^{10} or more particles will contain a number of spontaneously occurring mutant particles including mutants that attack additional hosts (extended-host-range mutants: see [10, 1045, 406]). All that is needed for the selection of such mutants are sensitive host cells (host cells having suitable phage receptors and no inimicable endonucleases). Just as a high-titer stock of phage is a source of mutant particles, so a dense culture of bacteria is a source of phage-resistant bacterial mutants (mutants which fail to make a particular receptor). As is well known, a high-titer stock of phage is also a powerful tool for eliminating the predominant sensitive members of the bacterial indicator population and selecting out the rare resistant (mutant) cells. Cultures of these resistant clones in turn can be used in a reversed procedure for selecting host range mutants of phage. For isolating new mycobacteriophages from the wild, Grant (464) has designed membrane filter sandwiches of specific mycobacteria, on which phages are trapped prior to enrichment through incubation with the desired host strain.

In many phage-host systems, the host cell has the capacity to destroy the incoming phage DNA through the agency of an endonuclease located near the cytoplasmic membrane (50, 172). Such host cells also have the capacity to modify entering DNA that has escaped the cell's protective endonuclease. When occasional phage (DNA) genomes become so modified, stocks of them can be prepared which bring about the lysis of a previously unsusceptible (restricting) bacterium. Thus, the combination of host restriction and modification also offers a means of expanding the lytic potential of a particular phage and, therefore, its host range. The authors, in collaboration with Pollice, have found restricting and modifying systems to be common among mycobacteria and nocardias. This was to be expected, since such endonucleases and DNA-modifying systems are common among corynebacteria (96, 685). Millman (821) was probably dealing with restriction and modification in 1958 when his 607-grown phage failed to lyse either $H_{37}Rv$, $H_{37}Ra$, or BCG, whereas $H_{37}Ra$-grown phage did lyse both the *M. tuberculosis* and the *M. bovis* strains. Nordström and Grange (883) have exploited the phage-modifying capacities of *M. fortuitum* in elaborating a phage-typing system for that species of mycobacterium. Sometimes the process of apparent modification (adaptation) may actually result from recombination between superinfecting phage and the resident phage genome, which is itself induced by the superinfecting phage to assume the vegetative phenotype (541; for the operation of such a recombinational system in the CMN group, see [517]).

The existence of the lysogenic state offers additional means for adding specificity to phage-typing schemes. Sometimes, on old slants of lysogenic strains, areas of lysis appear. These result from the lytic activity of virulent mutants of the carried prophage. Such mutants have lost the capacity to respond to repressor. They, too, can be used for expanding a typing scheme.

Laboratories given to phage typing are usually dealing with two kinds of lysis: (i) nonproductive lysis, murolysis. In many phage stocks there is an accumulation of murolytic enzymes that lyse bacterial cells by destroying portions of their cell walls. This nonproductive lysis is unrelated to phage multiplication. Such enzymatic lysis was used by Millman for preparing protoplasts of *M. tuberculosis* and *M. bovis* (821 see also [990]). (ii) Productive lysis. The specific phage-induced lysis involves phage replication, so beautifully illustrated at the cellular level by the pictures of Kölbel (a la Kellenberger, 606) showing the growth of mycobacteriophage Bo5 in *Mycobacterium vaccae* (629), where the destruction of the cell coincides with the liberation of a burst of newly formed bacteriophages. Each of these two mechanisms of lysis can offer useful information in the identification of unknown mycobacteria.

With some understanding of phage-host interaction, the preceding devices for tailoring the specificity of phages as to host receptors, host endonucleases, lysogenic immunity, permissive temperatures for replication, plaque

morphology (including halos versus no halos [660]), and lysis due to cell wall destruction versus productive lysis due to phage replication can be exploited for the phage typing of mycobacteria. Probably enough mycobacteriophages already exist for accomplishing the job. Much effort must be expended, however, for producing a workable collection of typing phages. Phage stocks are not indefinitely stable. Practicing laboratories will need to get used to retitering stocks almost weekly and to substitute actual numbers of plaque-forming particles for RTD (routine test dilution, 1109). Growing so-called slow-growing mycobacteria such as $H_{37}Rv$ in shake culture so that actively growing cells are available needs to become routine. In our laboratory plaques can be read on $H_{37}Rv$, so prepared, in 2 days, and that is the length of time it takes corynebacteriophages to develop plaques on the Park-Williams number 8 strain of *C. diphtheriae*. Mycobacteria grown on Tween-supplemented media are freed of the Tween (by dilution or washing) prior to using them in phage assays. Thus, plenty of evidence exists to indicate that, among mycobacteriophages, there is an adequate source of material for designing a system for the definitive typing of mycobacteria. Although the WHO committee has attributed much of their difficulties with phage typing of mycobacteria to the "polyvalency" of mycobacteriophages, there seems, in fact, little or no justification for such an excuse.

Phage Typing of Mycobacteria such as *M. tuberculosis* and *M. fortuitum*

Baess (79) has shown the possibilities of bacteriophage typing for sorting the strains of *M. tuberculosis* and, in so doing, has reviewed the efforts in this same direction of earlier workers, including Froman et al. (407), Takeya and associates (1137), Murohashi and his group (866), and Sugita et al. (1106). The materials and methods section of Baess' paper clearly spells out how one uses existing phage stocks for locating host range mutants. She shows that the phage type of *M. tuberculosis* is, as with other bacteria, a property of real constancy and as stable as any other inherited trait. Her conclusions as regards the usefulness of phage typing in the epidemiology of tuberculous disease attributable to *M. tuberculosis* agrees with the findings of Tokunaga et al. (1157). Baess' discussion of the pitfalls of phage typing of *M. tuberculosis* indicates the importance to such work of an understanding of the methods of bacterial virology, including single-plaque isolation, adsorption experiments, production of stocks of high titer, etc. Bates and Mitchison (105; see also [1201] with regard to phage type of strains of *M. tuberculosis* from Hungary) have examined the geographic distribution of *M. tuberculosis* according to phage types. Their data indicate that (i) *M. tuberculosis* from different regions tended to be different phage types; (ii) phage type was a constant property, and (iii) the mutation to drug resistance did not involve a change in phage type. Phage type as a stable genetic marker seems well established for *M. tuberculosis*. Stable markers can be of use in such problems of epidemiology as deciding whether reinfection tuberculosis is endogenous (1091) or exogenous. Both modes certainly operate. Raleigh and Wichelhausen (957) have reported a third clinical episode of pulmonary tuberculosis in a patient in whom the final infecting strain of *M. tuberculosis* was distinct as to drug resistance, urease production, and phage type. A close associate of the patient was infected with *M. tuberculosis* of this same phage type. If one assumes that the frequency of the mutations in drug resistance pattern, urease activity, and phage type are as high as 10^{-7} each, then the chance that the strain causing the final infection was directly related to that causing the second infection seems very remote (i.e., 10^{-21}).

Baess and Bentson, extending the earlier efforts of Käppler (581), Rodda (989), Tokunaga and associates (1156), and Juhasz and Bönicke (566), have offered a basis for the development of a phage-typing scheme that could distinguish *M. phlei*, *M. smegmatis*, *M. fortuitum*, *M. vaccae* and, by extension, other rapidly growing mycobacteria (81). Among the additional markers investigated were (i) growth at 37, 45, and 52°C, (ii) ability to deamidate some of 13 amides, and (iii) ability to ferment some of 12 carbohydrates. Nordström and Grange have employed host-induced modification for developing a set of phage capable of discriminating differences in strains of *M. fortuitum* (883). These authors also have established a correlation between inositol utilization by strains of *M. fortuitum* and their susceptibility to a particular phage, BK4 (462). Gunnels and Bates have shown that "typing" phages can be used for identifying *M. xenopi* (478).

Host Cell Receptors and Mycobacteriophage-Induced Receptor-Destroying Enzymes

Imaeda and San Blas found that the receptors for a phage, GS-7, were located in a lipoidal component of the cell wall of *M. smegmatis* 607 (533). The glycolipids and peptidoglycolipids are surface components of the mycobacterial cell, and the first act of the infecting virus is to

fix to the surface. One would expect to encounter mycobacteriophages capable of attaching to a variety of mycobacterial surface components. The virus does not distinguish isolated receptors from in situ receptors. Some viruses, after adsorption to isolated receptors, "inject" their DNA into the milieu. The fixation to isolated receptors effectively eliminates the infectivity of the virus. Thus, the receptor suitability of a cell component can be demonstrated by the same general techniques used for demonstrating the neutralizing capacity of antiviral antibody. The mixing of small amounts of preparations of mycosides C (see section on Peptidoglycolipids) with phage D4 effectively neutralized the infectivity of it but not that of unrelated phages. Castelnuovo et al. recovered a glycolipid (lacking any ninhydrin-positive component) from extracts of the cell envelopes of *M. phlei* which inactivates phage *phlei*, causing a drop in infectivity of 4 logs in 60 min (221). Once a number of specific, distinct, and chemically characterized receptor substances become available they can be used to classify the receptor apparatus of a particular phage simply by testing their capacity to neutralize the infectivity of that phage. This would enable laboratories to gain such information without resorting to the sophisticated chemistry needed for characterizing mycobacterial receptor lipids.

Bacteriophages of many classes of bacteria have associated with their replication the synthesis of enzymes capable of *degrading* those sites on the bacterial surface to which the phage attaches. Such catalysts can conveniently be referred to as receptor-destroying enzymes (RDE; 208). Although the original designation, RDE, referred to the destruction of neuraminidase-sensitive receptors on mammalian cells (207), the term is here used to cover all receptor-destroying enzymes. The plaques of phages that produce RDE usually are surrounded by a halo. Gratia (465) long ago showed that from the center of such plaques one could recover phage, whereas from the zone of the halo or beyond no phage was to be found. In the lyses that produced the phages at the center of the plaque, RDE was also produced; the enzymes then diffused to the edge of the halo, changing the surface properties of the bacterial cells.

Such bacteria, lacking receptors, are phenotypically resistant to the bacteriophage responsible for receptor-destroying activity. In the CMN group, it is sometimes difficult to see the halos of plaques because the amount of substrate produced may so overwhelm the enzyme that no optical difference is apparent between the bacteria within the zone of RDE and those beyond. This matter can be obviated by allowing the phages and bacteria to interact prior to plating out in a lawn of bacteria. The concentration of bacteria should be minimal. After the plaques have developed, a second lawn of sensitive bacteria is layered over the first. Lysis proceeds, and the accumulated, plus current, yields of RDE produce a visible halo. The halo-producing property of phage Bo1 growing on *M. smegmatis* 607 has been documented by Kraiss et al. (660). Pictures of halo-forming corynebacteriophages are illustrated on p. 406 of reference 96. A collection of RDE should be of great value to those laboratories devoted to the study of the chemistry of mycobacterial lipids.

Jones and David (562) have made a preliminary report of the production (during one step of growth of phage D29 on *M. smegmatis* 607) of a lipase that liberates lauric acid from Tween 20. Similar lipase activity was found in cells of *M. fortuitum* infected with D29, but not in uninfected cells of either *M. fortuitum* or *M. smegmatis* 607. A function for the lipase in the phage-infected cell has not yet been sought.

MYCOBACTERIOCINS

Although low-molecular-weight bacteriocins (174) produced by mycobacteria and inhibitory for other mycobacteria have been known since 1958 (851), these dialyzable antibiotics have not yet been used in a system for ordering relationships among mycobacteria. Additional mycobacteriocins have been described (9, 1136), and Takeya and Tokiwa have had some success with preliminary sorting of *M. tuberculosis* (nine strains) with 11 bacteriocins (1136). The typing of new isolates from the same patients over a period of time showed the infecting types to remain the same. We have encountered no reports of high-molecular-weight bacteriocins (174) active on mycobacteria. Since the high-molecular-weight bacteriocins occur for a wide variety of bacteria, one would expect some to be found which are active on mycobacteria. Eventually, it should be possible to combine phages and bacteriocins into one typing scheme.

SOME ABBREVIATIONS USED IN THE TEXT

ACP	Acyl carrier protein
D-Ala	D-Alanine
L-Ala	L-Alanine
AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
B cells or B lymphocytes	Lymphoid stem cells which have had an extrathymic maturation

BCG	*M. bovis* strain BCG, bacille Calmette Guérin
BCG-itis	Regional lymphadenitis sometimes resulting from vaccination
BGG	Bovine gamma globulin
BSA	Bovine serum albumin
C'3 or C3	The third component of complement (C1-9)
CBH	Cutaneous basophil hypersensitivity
CCB	Competency for Clearing Bacilli test
CF	Cord factor(s), dimycolates of trehalose
CFA	Complete Freund adjuvant
CL	Cardiolipin(s)
CMI	Cell-mediated immunity
CMN	*Corynebacterium, Mycobacterium, Nocardia* group
CoA or CoASH	Coenzyme A
Con A	Concanavalin A
CPTA	2-(4-Chlorophenylthio)-triethylamine hydrochloride
CW	Cell wall
CWS	Cell wall skeleton: residual cell walls (one-third of starting material) obtained following freeing of protein with proteinase and of free lipids with neutral organic solvents (69)
CY	Cyclophosphamide
DAP (DPM)	Diaminopimelic acid
DH	Delayed hypersensitivity
DNA	Deoxyribonucleic acid
DNFB	Dinitrofluorobenzene
DMAPP	3,3-Dimethylallyl pyrophosphate
DS 500	Dextran sulfate 500
EDTA	Ethylenediaminetetraacetic acid
EM	Electron micrograph
EPP	Erythropoietic porphyria
ETP	Electron transport particles
ETZ	Electron-transparent zone
FA	Fatty acid
Fab or Fab fragment, two such fragments	Consisting of one heavy and one light chain, each containing one antibody combining site. Are derived by papain hydrolysis as in following.
Fc or Fc fragment	Crystallizable fragment obtained by papain hydrolysis of molecules of immunoglobulin: e.g., IgG Fc consists of C-terminal halves of two heavy chains linked by disulfide bonds (molecular weight, 50,000). Has no antibody activity, can fix to complement.
ft-c	Footcandles
FAS I	Fatty acid synthetase type I
FAS II	Fatty acid synthetase type II
FBG	Foreign body-type granuloma
FMN ($FMNH_2$)	Flavin mononucleotide (reduced form); same as riboflavin phosphate
G+C	Guanine + cytosine content
GDP-man	Guanosine diphosphomannose
GIF	Growth inhibitory factor
Gln	Glutamine
D-Glu	D-Glutamic acid
GlucNAc	*N*-acetylglucosamine
$H_{37}Ra$	*M. tuberculosis* attenuated strain derived from $H_{37}Rv$
$H_{37}Rv$	*M. tuberculosis*, standard virulent strain. For origins see text.
HEF	Heptane-soluble fatty acids
HG	Hypersensitivity-type granuloma
HI	Humoral immunity
HSA	Human serum albumin
ID	Immunodiffusion
IFA	Incomplete Freund adjuvant
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPP	Isopentenyl pyrophosphate
IU	International test unit
KLH	Keyhole limpet hemocyanin
KTC	Killer T cell (lymphocyte)
LMF	Lymphocyte mitogenic factor
LPS	Lipopolysaccharide from gram-negative bacteria
MAP	Murine acid phosphatase
MER	Methanol-extracted residue of cells of BCG
MER-FA	MER in IFA
MF	Millipore filter
MGLP	Methylglucose-containing lipopolysaccharides
MIF	Migration inhibition factor
MMP	3-*O*-methyl mannose polysaccharide
MurNAc	*N*-acetylmuramic acid
MurNGl	*N*-glycolylmuramic acid
myc RNA	Ribosomal fractions of (from) mycobacteria
NAD^+ (NADH)	Nicotinamide adenine dinucleotide (reduced form)
$NADP^+$ (NADPH)	Nicotinamide adenine dinucleotide phosphate (reduced form)
OT	Old tuberculin
OTZ	Outer transparent zone
PCA	Passive cutaneous anaphylaxis
PE	Phosphatidylethanolamine
PEc	Peritoneal exudate cells
PHA	Phytohemagglutinin
Phe I	Phenotype I, an in vivo phenotype
Phe II	Phenotype II, an in vitro phe-

	notype
PI-Man$_x$	Phosphatidylinositol oligomannoside
PL	Phospholipid
PMN	Polymorphonuclear leukocytes
poly(A)·poly(U)	Polyadenylic acid–polyuridylic acid
PPD	Purified protein derivative
PPD-S	PPD from *M. tuberculosis*
RCF	Reference culture filtrates
RDE	Receptor-destroying enzymes
REC	Reticuloendothelial cells
RNA	Ribonucleic acid
RTD	Routine test dilution
607	*Mycobacterium smegmatis* ATCC 607
SAFA	Soluble-antigen fluorescent antibody
SEA	*Shistosoma* egg antigen
SL	Sulfolipid
SRBC	Sheep erythrocytes
T	Lymphoid stem cells which have had an association with thymus or thymosin
TLC	Thin-layer chromatography
ts	Temperature-sensitive mutants
2D-IEP	Two-dimensional immunoelectrophoresis
UDP	Uridine 5′-diphosphate
UV	Ultraviolet light
WHO	World Health Organization
WSA	Water-soluble adjuvant
WSA-FA	WSA substituted for mycobacteria in CFA, i.e., WSA plus IFA

SUMMARY

The Mycobacterial Cell

Mycobacteria are gram-positive, nonmotile, nonsporeforming, pleomorphic rods. The cellular morphology of several species is not easily distinguished from that of other members of the CMN group, *Corynebacterium* and *Nocardia*. Some mycobacteria show a tendency to form aerial filaments: they range from nonfilamentous *M. tuberculosis* to *M. fortuitum*, with limited filamentation and fragmentation, to filamentous *M. farcinogenes*.

Mycobacterium can be distinguished from the genera *Corynebacterium* and *Nocardia* on the basis of certain ropelike structures often found as a superficial "layer" (see Fig. 6, 25, and 26). Mycobacteria exhibit an acid-fastness distinct from the acid-fastness of *Corynebacterium*, *Nocardia*, bacterial spores, fungal spores, human sperm, etc. Mycobacterial acid-fastness is lost upon removal of the ropelike structures. Mycobacterial acid-fastness is compounded from two events: (i) the formation of arylmethane-mycolates between fuchsin and the mycolic acids of the ropelike structures and (ii) the trapping of intracellular fuchsin by the dye-complexed ropelike structures in conjunction with other components of the outer cell wall. Mycobacteria may be non-acid-fast (and generally chromophobic) when they produce substances that cover up their ropelike structures.

Ultrastructure. Electron micrographs of actively growing mycobacteria, negatively stained, freeze fractured, metal shadowed, or ultrathin sectioned and stained, indicate that beyond the cytoplasmic membrane there is a rigid layer, the murein, upon which is superposed three discernible layers: L_3, L_2, and L_1 (Fig. 4 and 5). At the macromolecular level, these are *apparent* (only) layers deriving from interconnecting structures stretching outward from the basement muramyl peptidoglycan, as predominantly arabinogalactan-mycolate with some arabinomannan and some peptidolipid, the ropelike structures of peptidoglycolipid (L_2) terminating in ribbons at the cell surface (L_1). Interspersed in these same outer areas are mycolates of trehalose, including sulfolipids and cord factors.

The average size of the DNA of the mycobacterial nucleoid is around 3.0×10^9 daltons, and its G+C content is about 65%. Sections of mycobacterial cells in logarithmic growth exhibit fibrillar DNA associated with mesosomal extensions of the cytoplasmic membrane system. The cytoplasm is rich with densely packed ribosomes. Cells in which metabolism is not at maximum rate contain lipoidal bodies, metachromatic granules, and small, dense granules of unknown composition. The ultrastructure of the mycobacterial cell (see foregoing) indicates that it is capable of a variety of biosynthetic processes.

Biosyntheses. *Mycobacterium*, *Corynebacterium*, and *Nocardia* are remarkable for the lipids they synthesize. *M. phlei* (and *C. diphtheriae*) possesses a stable, multienzyme complex, FAS I, carrying its own ACP, which is capable of catalyzing the synthesis of long-chain FAs as follows:

$$CH_3\text{-}CO\text{-}SCoA + n HOOC\text{-}CH_2\text{-}CO\text{-}SCoA + x TPNH + y DPNH + (x + y)H^+ \rightarrow CH_3\text{-}[CH_2\text{-}CH_2]_n\text{-}CO\text{-}SCoA + n CoASH + n CO_2 + x TPN^+ + y DPN^+ + n H_2O$$

(143). In addition, this complex catalyzes the elongation of acyl-CoA derivatives with carbon chains up to C_{20}. Special mycobacterial methylated polysaccharides and lipopolysaccharides are capable of markedly activating FAS I. *M. phlei*, also, contains a FAS II (malonyl-CoA incorporation), which requires added ACP.

Among the major phospholipids of mycobacteria are PEs, diphosphatidyl glycerides, and myoinositol phospholipids, including a phosphatidylinositol pentamannoside. When provided free FA as palmitate or in serum or from lipids available in tissue, mycobacteria produce visible intracellular globules of lipid, probably stored as di- and triacylglycerides (see Fig. 13). The acylating capabilities of the CMN group are also rapidly turned on in the presence of excess glucose, and acylglucose is produced.

The carotenoid pigments of mycobacteria, exploited for taxonomic purposes, are synthesized constitutively by some strains, inducibly by some, and not at all by others (see Fig. 16 and 17). The protection these polyene compounds afford the mycobacterial cell relates to the sensitivity of mycobacteria to toxic photoproducts initiated by white (perhaps, also UV) light. Protoporphyrin IX or undesignated flavins or all of these appear required for the light-induced formation of these toxic substances. Whether respiratory quinones are destroyed upon exposure of mycobacteria to light, as in *Sarcina lutea*, has not been determined. Menaquinones *are* important respiratory molecules in mycobacteria.

Taxonomy. The antigenic analysis of mycobacteria is, after 50 years of intensive effort, still in its infancy. Valuable advances have been made regarding the serology of *M. avium*. As is so often the case, no correlation between virulence and serological type has been established. Soluble antigens of mycobacteria have been examined by a variety of means, often with interesting results. Among these have been attempts to establish relatedness through immunodiffusion patterns. The interpretation of such patterns is markedly limited by the lack of either well-characterized antigens or well-characterized antibodies. In this connection, there has been generally admitted a need for meaningful reference antigens and antisera.

The delineation of species within *Mycobacterium* is accomplished through the use of a dozen or more tests including: presence or absence of enzymes such as catalase and arylsulfatase, rates of growth, capacity to produce carotenoid pigments, the secretion of niacin, sensitivity to sodium chloride, the reduction of tellurite, and the hydrolysis of polyoxyethylene sorbitan monooleate.

Mycobacteriophages and Genetics of Mycobacteria

A number of mycobacteriophages exhibiting a wide range of ultrastructure are described herein (Fig. 29 through 32) and await exploitation for the taxonomy and the genetics of *Mycobacterium*. Genetic recombination has been effected between two strains of *M. smegmatis*.

Interaction with the Animal Host

Macrophages. Many mycobacteria are capable of surviving in macrophages of animal hosts. Apparently, the phagosome-lysosome fusion associated with the killing of bacteria is not very evident in macrophages that have engulfed, for example, *M. tuberculosis* $H_{37}Rv$. Even when such fusion has been induced in normal macrophage, $H_{37}Rv$ is not killed. Those mycobacteria that survive in macrophages of animal hosts assume a phenotype (Phe I) differing in distinct ways from their homologous in vitro (Phe II) phenotype: e.g., in metabolizing certain energy sources, in producing detectable sulfolipid, or in immunogenicity. "Clones" of Phe I of *M. tuberculosis* occur principally in tuberculous granulomas, adventitious colonies from the reticuloendothelial system, consisting of macrophages gorged with tubercle bacilli, epithelioid cells, and multinucleated giant cells, all surrounded by fibroblasts and lymphocytes. New macrophages constantly enter the granulomas, phagocytosing bacilli and fragments from dying macrophages. There is evidence to support the assumption that in states of specific delayed hypersensitivity related to PPD, there is an enhanced activity of macrophages within granulomas. A factor found in BCG-immune serum (rabbit) induces giant cell formation in macrophages from nonimmune rabbits when incubated with heat-killed BCG. *Nocardia braziliensis* can be substituted for BCG in this induction, suggesting that perhaps other members of the CMN group might function similarly.

Immune response. In experimental infections with *M. tuberculosis* in guinea pigs and in human tuberculosis, there is a turning on of both "humoral" (HI) and "cellular" immunity (DH and CMI), all mediated by cells. Folklore has long held that tubercular individuals are less prone to the common run of infections than nontuberculars. The laboratory contribution to this opinion derives from the establishment of the adjuvant action of mycobacteria. Living mycobacteria administered to animals along with antigen X bring about an enhancement in the production of antibodies versus antigen X. Although dead mycobacteria cannot substitute for living mycobacteria as adjuvants, dead mycobacteria in a water-in-oil emulsion (CFA) are well-known adjuvants. Immunization with antigen X in CFA can lead to (i) enhanced production of antibody versus antigen X and (ii) DH

versus antigen X. (iii) When administered with certain tumor antigens, Y, or when said tumor antigens, Y, are administered with CFA, there develop lymphocytes cytotoxic in vitro for Y tumor cells. (iv) In addition, adjuvant arthritis and general adjuvant disease may develop in a number of animals receiving CFA. Variations on the time of administration and amounts of doses of CFA can suppress the immune response. Under certain conditions the full development of DH may require suppressor B cells. When CFA is administered with two distinct antigens, the outcome of the antibody response may be altered: the phenomenon, antigenic competition. Various side effects of CFA make it unsuitable for immunization of humans. Living BCG and living *M. microti* are the mycobacterial vaccines most studied in man. They, too, in a small number of subjects, give untoward reactions. Many concerned with developing mycobacterial vaccines hope to find mycobacterial components capable of immunizing and/or acting as adjuvants without producing damaging side effects. In a number of human subjects suffering from chronic tuberculosis, as well as in about 25% of rabbits suffering from experimental chronic infections including tuberculosis, there develop autoantibodies that are cytotoxic for the animals' cells growing in tissue culture.

Virulences of mycobacteria are no more and no less complicated than virulences of *Histoplasma, Leishmania,* or *Candida*. Virulence in *M. avium* appears to be associated with a particular surface antigen. Virulence in *M. tuberculosis* is associated with those molecular species that make possible "cording" of colonies. In rabbits and man, there are also genetic factors of the host that affect the outcome of infections. The extent to which these relate to antigens common to host and bacterium is just now being questioned.

Components inducing specific host responses. Fruitful results in localizing the biological activity(ies) of mycobateria to certain subcellular components follow.

(i) *Cord factor*. The dimycolate(s) of trehalose, cord factor(s), (a) are toxic for mitochondrial membranes, (b) when conjugated to a suitable carrier serve as functional haptens, and (c) exhibit adjuvant activity when administered (as a mixture designated P3) with PPD. Toxicity of these dimycolates depends upon certain free hydroxyl groups on the molecules of trehalose. A related sulfur-containing molecule, mycobacterial sulfolipid, appears capable of enhancing the toxicity of cord factor.

(ii) *Nonmurein peptides, tuberculin-active peptides*. Many mycobacterial elicitins (tuberculin-active peptides) appear to originate from the mycobacterial cell wall. Tuberculin remains the most widely studied such mycobacterial product. As OT and various commercially available PPDs, it is comprised of at least five distinct elicitins. Although PPD itself does not sensitize animals to a state of tuberculin positivity, PPD plus poly(A)·poly(U) does effect sensitization.

(iii) *Muramic acid-linked dipeptides*. The sugar dipeptide, *N*-acetylmuramyl-L-alanyl-D-isoglutamine, administered in mineral oil with antigen X, brings about an enhancement of specfic antibody synthesis and the development of DH to antigen X.

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Note Added *In Press*

The substitution of "et al." for names of investigators referred to herein results from a policy of *Bacteriological Reviews*.

LITERATURE CITED

1. **Aaronson, C. M., and C. H. Park.** 1974. Sporotrichoid infection due to *Mycobacterium marinum:* lesion exacerbated by corticosteroid infiltration. South. Med. J. 67:117-118.
2. **Abrahams, E. W.** 1970. "Original mycobacterial sin." Tubercle 51:316-321.
3. **Acharya, P. V. N., and D. S. Goldman.** 1970.

Chemical composition of the cell wall of the H37Ra strain of *Mycobacterium tuberculosis*. J. Bacteriol. 102:733-739.
4. **Acharya, P. V. N., M. Senn, and E. Lederer.** 1967. Sur la présence et la structure de mycolates d'arabinose dans les lipides liés de deux souches de Mycobactéries. C.R. Acad. Sci. Ser. C 264:2173-2176.
5. **Adam, A., R. Ciorbaru, F. Ellouz, J.-F. Petit, and E. Lederer.** 1974. Adjuvant activity of monomeric bacterial cell wall peptidoglycans. Biochem. Biophys. Res. Commun. 56:561-567.
6. **Adam, A., R. Ciorbaru, J.-F. Petit, and E. Lederer.** 1972. Isolation and properties of a macromolecular, water-soluble, immunoadjuvant fraction from the cell wall of *Mycobacterium smegmatis*. Proc. Natl. Acad. Sci. U.S.A. 69:851-854.
7. **Adam, A., R. Ciorbaru, J.-F. Petit, E. Lederer, L. Chedid, A. Lamensans, F. Parant, M. Parant, J. P. Rosselet, and F. M. Berger.** 1973. Preparation and biological properties of water-soluble adjuvant fractions from delipidated cells of *Mycobacterium smegmatis* and *Nocardia opaca*. Infect. Immun. 7:855-861.
8. **Adam, A., J.-F. Petit, J. Wietzerbin-Falszpan, P. Sinay, D. W. Thomas, and E. Lederer.** 1969. L'acide *N*-glycolyl-muramique, constituant des parois de *Mycobacterium smegmatis:* identification par spectrometrie de masse. FEBS Lett. 4:87-92.
9. **Adamek, L., L. Trnka, P. Mison, and M. Gutova.** 1968. Hemmstoffe vom Bakteriocin-Typus bei schnell wachsenden saprophytischen Stammen der Mycobakterien. Beitr. Klin. Tuberk. Spezifischen Tuberk. Forsch. 138:51-55.
10. **Adams, M. H.** 1959. Bacteriophages, p. 592. Interscience Publishers, New York.
11. **Adams, R. M., J. S. Remington, J. Steinberg, and J. S. Seibert.** 1970. Tropical fish aquariums. A source of *Mycobacterium marinum* infections resembling sporotrichosis. J. Am. Med. Assoc. 211:457-461.
12. **Aebi, A., J. Asselineau, and E. Lederer.** 1953. Sur les lipides de la souche humaine "brévannes" de *Mycobacterium tuberculosis*. Bull. Soc. Chim. Biol. 35:661-684.
13. **Affronti, L. F., E. H. Fife, and L. Grow.** 1973. Serodiagnostic test for tuberculosis. Am. Rev. Respir. Dis. 107:822-825.
14. **Affronti, L. F., and F. B. Seibert.** 1965. Mycobacterial antigen fractionations. Some early investigations of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 92(part 2): 1-8.
15. **Affronti, L. F., G. L. Wright, Jr., and M. Reich.** 1972. Characterization and comparison of mycobacterial antigens by discontinuous pore gradient gel electrophoresis. Infect. Immun. 5:474-481.
16. **Aho, K., E. Brander, and T. Estola.** 1973. Agglutinins against group III atypical mycobacteria. Acta Pathol. Microbiol. Scand. Sect. B 81:589-592.
17. **Akamatsu, Y., and J. H. Law.** 1968. Enzymatic synthesis of 10-methylene stearic acid and tuberculostearic acid. Biochem. Biophys. Res. Commun. 33:172-176.
18. **Akamatsu, Y., and J. H. Law.** 1970. Enzymatic alkylenation of phospholipid fatty acid chains by extracts of *Mycobacterium phlei*. J. Biol. Chem. 245:701-708.
19. **Akamatsu, Y., and J. H. Law.** 1970. The enzymatic synthesis of fatty acid methyl esters by carboxyl group alkylation. J. Biol. Chem. 245:709-713.
20. **Akamatsu, Y., and S. Nojima.** 1965. Separation and analyses of the individual phospholipids of mycobacteria. J. Biochem. 57:430-439.
21. **Akamatsu, Y., Y. Ono, and S. Nojima.** 1966. Phospholipid patterns in subcellular fractions of *Mycobacterium phlei*. J. Biochem. 59:176-182.
22. **Akamatsu, Y., Y. Ono, and S. Nojima.** 1967. Studies on the metabolism of phospholipid in *Mycobacterium phlei*. 1. Difference in turnover rates of individual phospholipids. J. Biochem. 61:96-102.
23. **Allen, S. H. G., B. F. Jacobson, and R. Stjernholm.** 1964. Biocytin as a constituent of methylmalonyl-oxaloacetic transcarboxylase and propionyl CoA carboxylase of bacterial origin. Arch. Biochem. Biophys. 105:494-500.
24. **Allison, A. C.** 1973. Effects of adjuvants on different cell types and their interactions in immune responses, p. 73-99. *In* G. E. W. Wolstenholme and J. Knight (ed.), Immunopotentiation. Ciba Foundation Symposium 18 (new series). Associated Scientific Publishers, Amsterdam.
25. **Allison, A. C., and A. J. S. Davies.** 1971. Requirement of thymus-dependent lymphocytes for potentiation by adjuvants of antibody formation. Nature (London) 233:330-332.
26. **Allwood, G. G., and G. L. Asherson.** 1971. Depression of delayed hypersensitivity by pretreatment with Freund-type adjuvants. II. Mechanism of the phenomenon. Clin. Exp. Immunol. 9:259-266.
27. **Alsaadi, A-I., and D. W. Smith.** 1973. The fate of virulent and attenuated mycobacteria in guinea pigs infected by the respiratory route. Am. Rev. Respir. Dis. 107:1041-1046.
28. **Alsop, D. G.** 1972. The Bairnsdale ulcer. Aust. N. Z. J. Surg. 41:317-319.
29. **Altman, L. J., L. Ash, R. C. Kowerski, W. W. Epstein, B. R. Larsen, H. C. Rilling, F. Muscio, and D. E. Gregonis.** 1972. Prephytoene pyrophosphate. New intermediate in the biosynthesis of carotenoids. J. Am. Chem. Soc. 94:3257-3259.
30. **Alvarez and Tavel.** 1885. Recherches sur le bacille de Lustgarten. Arch. Physiol. Norm. Pathol. 6:303-321.

31. **Amar, C., and E. Vilkas.** 1973. Isolement d'un phosphate d'arabinose àpartir des parois de *Mycobacterium tuberculosis*, H37Ra. C.R. Acad. Sci. Ser. D 277:1949-1951.
32. **Amar-Nacasch, C., and E. Vilkas.** 1969. Étude des parois d'une souche humaine virulente de *Mycobacterium tuberculosis*. 1. Préparation et analyse chimique. Bull. Soc. Chim. Biol. 51:613-620.
33. **Amar-Nacasch, C., and E. Vilkas.** 1970. Étude des parois de *Mycobacterium tuberculosis*. II. Mise en évidence d'un mycolate d'arabinobiose et d'un glucane dans les parois de *M. tuberculosis* H37Ra. Bull. Soc. Chim. Biol. 52:145-151.
34. **American Lung Association.** 1974. Diagnostic standards and classification of tuberculosis and other mycobacterial diseases, p. 41. American Lung Association, New York.
35. **Amos, H. E., B. W. Gurner, R. J. Olds, and R. R. A. Coombs.** 1967. Passive sensitization of tissue cells. II. Ability of cytophilic antibody to render the migration of guinea pig peritoneal exudate cells inhibitable by antigen. Int. Arch. Allergy Appl. Immunol. 32:496-505.
36. **Anderson, E. S.** 1957. The relations of bacteriophages to bacterial ecology, p. 189-217. *In* R. E. D. Williams and C. C. Spicer (ed.), Microbial ecology, 7th Symposium of the Society of General Microbiology. University Press, Cambridge.
37. **Anderson, E. S., and A. Felix.** 1953. The Vi type-determining phages carried by *Salmonella typhi*. J. Gen. Microbiol. 9:65-88.
38. **Anderson, J. S., M. Matsuhashi, M. A. Haskin, and J. L. Strominger.** 1965. Lipid-phosphoacetylmuramyl-pentapeptide and lipid-phosphodisaccharide-pentapeptide: presumed membrane transport intermediates in cell wall synthesis. Proc. Natl. Acad. Sci. U.S.A. 53:881-889.
39. **Anderson, R. G., H. Hussey, and J. Baddiley.** 1972. The mechanism of wall synthesis in bacteria. The organization of enzymes and isoprenoid phosphates in the membrane. Biochem. J. 127:11-25.
40. **Anderson, R. J.** 1939. The chemistry of the lipoids of the tubercle bacillus and certain other microorganisms. Fortschr. Chem. Org. Naturst. 3:145-202.
41. **Anderson, R. J.** 1943. The chemistry of the lipids of the tubercle bacillus. Yale J. Biol. Med. 15:311-345.
42. **Anderson, T. F.** 1953. The morphology and osmotic properties of bacteriophage systems. Cold Spring Harbor Symp. Quant. Biol. 18:197-203.
42a. **Andersson, L. C., H. Binz, and H. Wigzell.** 1976. Specific unresponsiveness to transplantation antigens induced by autoimmunisation with syngeneic, antigen-specific T lymphoblasts. Nature (London) 264:778-780.
43. **Ando, M.** 1973. Macrophage activation in tuberculin reactions of rabbits with primary BCG infection and reinfection. J. Reticuloendothel. Soc. 14:132-145.
44. **Ando, M., A. M. Dannenberg, Jr., and K. Shima.** 1972. Macrophage accumulation, division, maturation and digestive and microbicidal capacities in tuberculous lesions. II. Rate at which mononuclear cells enter and divide in primary BCG lesions and those of reinfection. J. Immunol. 109:8-19.
45. **Andrejew, A.** 1948. L'utilisation par oxydation de quelques substances azotées par le bacille de Koch. Ann. Inst. Pasteur Paris 74:464-477.
46. **Andrejew, A., and A. Renard.** 1968. Essai de séparation des activités catalasique et peroxydasique chez les mycobactéries. Ann. Inst. Pasteur Paris 115:3-10.
47. **Antoine, A. D., and B. S. Tepper.** 1969. Environmental control of glycogen and lipid content of *Mycobaterium phlei*. J. Gen. Microbiol. 55:217-226.
48. **Antoine, A. D., and B. S. Tepper.** 1969. Environmental control of glycogen and lipid content of *Mycobacterium tuberculosis*. J. Bacteriol. 100:538-539.
48a. **Anz, W., and G. Meissner.** 1972. Vergleichende Virulenzprüfungen am Huhn von transparenten und opaken Kolonien aus Stämmen der aviaren Mykobakteriengruppe verschiedener Serotypen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig. Reihe A 221:334-342.
49. **Anz, W., G. Meissner and U. Röder.** 1969. Serologische Untersuchungen an atypischen Mycobakterien mittels der Agglutinationsmethode. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. 211:530-550.
50. **Arber, W., and S. Linn.** 1969. DNA modification and restriction. Annu. Rev. Biochem. 38:467-500.
51. **Armstrong, J. A., and P. D. Hart.** 1975. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. J. Exp. Med. 142:1-16.
52. **Aronson, J. D.** 1926. Spontaneous tuberculosis in salt water fish. J. Infect. Dis. 39:315-320.
53. **Aronson, J. D.** 1929. Spontaneous tuberculosis in snakes; n. sp. *Mycobacterium thamnopheos*. J. Infect. Dis. 44:215-223.
54. **Asano, A., N. S. Cohen, R. F. Baker, and A. F. Brodie.** 1973. Orientation of the cell membrane in ghosts and electron transport particles of *Mycobacterium phlei*. J. Biol. Chem. 248:3386-3397.
55. **Asherson, G. L., and G. G. Allwood.** 1971. Depression of delayed hypersensitivity by pretreatment with Freund type adjuvants. I. Description of the phenomenon. Clin. Exp. Immunol. 9:249-258.
56. **Askenase, P. W., J. D. Haynes, D. Tauben, and R. De Bernardo.** 1975. Specific basophil

hypersensitivity induced by skin testing and transferred using immune serum. Nature (London) 256:52-54.
57. **Asselineau, C. P., C. S. Lacave, H. L. Montrozier, and J.-C. Promé.** 1970. Relations structurales entre les acides mycoliques insaturés et les acides inférieurs insaturés synthétisés par *Mycobacterium phlei*. Implications métaboliques. Eur. J. Biochem. 14:406-410.
58. **Asselineau, J.** 1961. Sur la composition des lipides de *Corynebacterium diphtheriae*. Biochim. Biophys. Acta 54:359-360.
59. **Asselineau, J.** 1966. The bacterial lipids. Holden-Day, Inc., San Francisco.
60. **Asselineau, J., H. Bloch, and E. Lederer.** 1954. Synthesis of cord factor-active substances. Biochim. Biophys. Acta 15:136-137.
61. **Asselineau, J., and M. Kato.** 1973. Chemical structure and biochemical activity of cord factor analogs. II. Relationships between activity and stereochemistry of the sugar moiety. Biochimie 55:559-568.
62. **Asselineau, J., and E. Lederer.** 1951. Sur la constitution chimique des acides mycoliques de deux souches humaines virulentes de *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 7:126-145.
63. **Asselineau, J., and E. Lederer.** 1955. Synthèses de substances ayant de structures analogues à celle du "cord factor": esters mycoliques de sucres; esters et amides mycoliques de sucres aminés. Bull. Soc. Chim. Fr., p. 1232-1240.
64. **Asselineau, J., and V. Portelance.** 1974. Comparative study of the free lipids of eight BCG daughter strains. Recent Results Cancer Res. 47:214-220.
65. **Audibert, F., L. Chédid, P. Lefrancier, and J. Choay.** 1976. Distinctive adjuvanticity of synthetic analogs of mycobacterial water-soluble components. Cell. Immunol. 21:243-249.
66. **Ayling, J., R. Pirson, and F. Lynen.** 1972. Participation of covalently linked fatty acyl coenzyme A products in the action of yeast fatty acid synthetase. Biochemistry 11:526-532.
67. **Azuma, I., M. Ajisaka, and Y. Yamamura.** 1970. Polysaccharides of *Mycobacterium bovis* Ushi 10, *Mycobacterium smegmatis*, *Mycobacterium phlei*, and atypical *Mycobacterium* P1. Infect. Immun. 2:347-349.
68. **Azuma, I., F. Kanetsuna, Y. Tanaka, M. Mera, Y. Yanagihara, I. Mifuchi, and Y. Yamamura.** 1973. Partial chemical characterization of the cell wall of *Nocardia asteroides* strain 131. Jpn. J. Microbiol. 17:154-159.
69. **Azuma, I., F. Kanetsuna, T. Taniyama, Y. Yamamura, M. Hori, and Y. Tanaka.** 1975. Adjuvant activity of mycobacterial fractions. I. Purification and *in vivo* adjuvant activity of cell wall skeletons of *Mycobacterium bovis* BCG, *Nocardia asteroides* 131 and *Corynebacterium diphtheriae* PW8. Biken J. 18:1-13.
70. **Azuma, I., S. Kishimoto, Y. Yamamura, and J.-F. Petit.** 1971. Adjuvanticity of mycobacterial cell walls. Jpn. J. Microbiol. 15:193-197.
71. **Azuma, I., A. Ohuchida, T. Taniyama, Y. Yamamura, K. Shoji, M. Hori, Y. Tanaka, and E. Ribi.** 1974. The mycolic acids of *Mycobacterium rhodochrous* and *Nocardia corallina*. Biken J. 17:1-9.
72. **Azuma, I., E. E. Ribi, T. J. Meyer, and B. Zbar.** 1974. Biologically active components from mycobacterial cell walls. I. Isolation and composition of cell wall skeleton and component P_3. J. Natl. Cancer Inst. 52:95-101.
73. **Azuma, I., D. W. Thomas, A. Adam, J-M. Ghuysen, R. Bonaly, J.-F. Petit, and E. Lederer.** 1970. Occurrence of *N*-glycolylmuramic acid in bacterial cell walls. Biochim. Biophys. Acta 208:444-451.
74. **Azuma, I., and Y. Yamamura.** 1962. Studies on the firmly bound lipids of human tubercle bacillus. I. Isolation of arabinose mycolate. J. Biochem. (Tokyo) 52:200-206.
75. **Azuma, I., and Y. Yamamura.** 1963. Studies on the firmly bound lipids of human tubercle bacillus. II. Isolation of arabinose mycolate and identification of its chemical structure. J. Biochem. (Tokyo) 53:275-281.
76. **Azuma, I., Y. Yamamura, and K. Fukushi.** 1968. Fractionation of mycobacterial cell wall. Isolation of arabinose mycolate and arabinogalactan from cell wall fraction of *Mycobacterium tuberculosis* strain Aoyama B. J. Bacteriol. 96:1885-1887.
77. **Azuma, I., Y. Yamamura, and A. Misaki.** 1969. Isolation and characterization of arabinose mycolate from firmly bound lipids of mycobacteria. J. Bacteriol. 98:331-333.
77a. **Bach, F. H., and J. J. van Rood.** 1976. The major histocompatibility complex-genetics and biology. N. Engl. J. Med. 295:806-813, 872-878, 927-936.
78. **Baer, H., and S. D. Chaparas.** 1963. The immunology and chemistry of tuberculin. I. The isolation of dialyzable and nondialyzable tuberculin-active components from unheated BCG culture filtrates. Am. Rev. Respir. Dis. 88:28-32.
79. **Baess, I.** 1969. Subdivision of *M. tuberculosis* by means of bacteriophages. With special reference to epidemiological studies. Acta Pathol. Microbiol. Scand. 76:464-474.
80. **Baess, I.** 1971. Report on a pseudolysogenic mycobacterium and a review of the literature concerning pseudolysogeny. Acta Pathol. Microbiol. Scand. Sect. B 79:428-434.
81. **Baess, I., and M. W. Bentzon.** 1969. Rapidly-growing mycobacteria. Susceptibility to bacteriophages, reactions in the amidase test, production of acid from carbohydrates, and growth at various temperatures. Acta

Pathol. Microbiol. Scand. 75:331-347.
82. Bailey, R. K., S. Wyles, M. Dingley, F. Hesse, and G. W. Kent. 1970. The isolation of high catalase *Mycobacterium kansasii* from tap water. Am. Rev. Respir. Dis. 101:430-431.
83. Bain, B., M. R. Vas, and L. Lowenstein. 1964. The development of large immature mononuclear cells in mixed lymphocyte cultures. Blood 23:108-116.
84. Baker, J. A. 1938. Light as a factor in the production of pigment by certain bacteria. J. Bacteriol. 35:625-631.
85. Baker, J. A., and W. A. Hagan. 1942. Tuberculosis of the Mexican platyfish (*Platypoecilus maculatus*). J. Infect. Dis. 70:248-252.
86. Baker, P. J., N. D. Reed, P. W. Stashak, D. F. Amsbaugh, and B. Prescott. 1973. Regulation of the antibody response to type III pneumococcal polysaccharide. J. Exp. Med. 137:1431-1441.
87. Baker, R. E., W. E. Hill, and C. L. Larson. 1973. Ribosomes of acid-fast bacilli: immunogenicity, serology, and in vitro correlates of delayed hypersensitivity. Infect. Immun. 8:236-244.
88. Baldwin, J. H. 1968. Pulmonary bovine tuberculosis in an owner and in his dairy herd. Cornell Vet. 58:81-87.
89. Baldwin, R. W., and M. V. Pimm. 1971. Influence of BCG infection on growth of 3-methylcholanthrene-induced rat sarcomas. Eur. J. Clin. Biol. Res. 16:875-881.
90. Ballou, C. E., and Y. C. Lee. 1964. The structure of a myoinositol mannoside from *Mycobacterium tuberculosis* glycolipid. Biochemistry 3:682-685.
91. Ballou, C. E., E. Vilkas, and E. Lederer. 1963. Structural studies on the myo-inositol phospholipids of *Mycobacterium tuberculosis* (var. *bovis*, strain BCG). J. Biol. Chem. 238:69-76.
92. Banerjee, R., R. Hall, and G. R. V. Hughes. 1970. Pulmonary *Mycobacterium fortuitum* infection in association with achalasia of the aesophagus. Case report and review of the literature. Br. J. Dis. Chest 64:112-118.
92a. Baram, P., L. Soltysik, and W. Condoulis. 1971. The *in vitro* assay of tuberculin hypersensitivity in *Macaca mulatta* sensitized with bacille Calmette Guerin cell wall vaccine and/or infected with virulent *Mycobacterium tuberculosis*. Lab. Anim. Sci. 21:727-733.
93. Bardana, E. J., Jr., J. K. McClatchy, R. S. Farr,and P. Minden. 1973. Universal occurrence of antibodies to tubercle bacilli in sera from non-tuberculous and tuberculous individuals. Clin. Exp. Immunol. 13:65-77.
94. Barker, D. J. 1971. Buruli disease in a district of Uganda. J. Trop. Med. Hyg. 74:260-264.
95. Barksdale, L. 1965. Discussion of Dr. B. R. Chatterjee's paper. Int. J. Lepr. 33:560-562.
96. Barksdale, L. 1970. *Corynebacterium diphtheriae* and its relatives. Bacteriol. Rev. 34:378-422.
97. Barksdale, L., and S. B. Arden. 1974. Persisting bacteriophage infections, lysogeny, and phage conversions. Annu. Rev. Microbiol. 28:265-299.
98. Barrow, G. I., and M. Hewitt. 1971. Skin infection with *Mycobacterium marinum* from a tropical fish tank. Br. Med. J. 2:505-506.
99. Bartholomew, W. R. 1968. Multiple catalase enzymes in two species of mycobacteria. Am. Rev. Respir. Dis. 97:710-712.
100. Bartlett, G. L., and B. Zbar. 1973. Adjuvants for a tumor-specific vaccine: *Bordetella pertussis* or mycobacterial cell walls attached to oil droplets. J. Natl. Cancer Inst. 50:1385-1390.
101. Bast, R. C., Jr., B. Zbar, T. Borsos, and H. J. Rapp. 1974. BCG and cancer. N. Engl. J. Med. 290:1413-1420, 1458-1469.
102. Basten, A., J. F. A. P. Miller, J. Sprent, and J. Rye. 1972. A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. J. Exp. Med. 135:610-626.
103. Basten, A., J. Sprent, and J. F. A. P. Miller. 1972. Receptor for antibody-antigen complexes used to separate T cells from B cells. Nature (London) New Biol. 235:178-180.
104. Basten, A., N. L. Warner, and T. Mandel. 1972. A receptor for antibody on B lymphocytes. II. Immunochemical and electron microscopy characteristics. J. Exp. Med. 135:627-642.
105. Bates, J. H., and D. A. Mitchison. 1969. Geographic distribution of bacteriophage types of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 100:189-193.
106. Batra, P. P. 1971. Mechanism of light-induced carotenoid synthesis in nonphotosynthetic plants, p. 47-76. *In* A. C. Giese (ed.), Photophysiology, vol. VI. Academic Press Inc., New York.
107. Batra, P. P., R. M. Gleason, Jr., and J. Jenkins. 1969. Mechanism of photo-induced and antimycin A-induced carotenoid synthesis in *Mycobacterium marinum*. Requirements for carotenogenesis and further evidence for protein synthesis following induction. Biochim. Biophys. Acta 177:124-135.
108. Batra, P. P., R. M. Gleason, Jr., and J. W. Louda. 1973. Cyclization of lycopene in the biosynthesis of β-carotene. Photochemistry 12:1309-1313.
109. Batra, P. P., T. L. Harbin, C. D. Howes, and S. C. Bernstein. 1971. A study of the relationship of structure and activity of antimycin A in the induction of carotenoid synthesis in *Mycobacterium marinum*. J. Biol. Chem. 246:7125-7130.
110. Batra, P. P., and H. C. Rilling. 1964. On the mechanism of photoinduced carotenoid synthesis: aspects of the photoinductive reaction. Arch. Biochem. Biophys. 107:485-492.

111. **Beaman, B. L., and J. Burnside.** 1973. Pyridine extraction of nocardial acid fastness Appl. Microbiol. 26:426–428.
112. **Beaman, B. L., K-S. Kim, M-A. Lanéelle, and L. Barksdale.** 1974. Chemical characterization of organisms isolated from leprosy patients. J. Bacteriol. 117:1320–1329.
113. **Beaman, B. L., K-S. Kim, M. R. J. Salton, and L. Barksdale.** 1971. Amino acids of the cell wall of *Nocardia rubra*. J. Bacteriol. 108:941–943.
114. **Beaman, B. L., and D. M. Shankel.** 1969. Ultrastructure of *Nocardia* cell growth and development on defined and complex agar media. J. Bacteriol. 99:876–884.
115. **Beck, A.** 1965. *Mycobacterium fortuitum* in abscesses of man. J. Clin. Pathol. 18:307–313.
116. **Bekierkunst, A., I. S. Levij, E. Yarkoni, E. Vilkas, A. Adam, and E. Lederer.** 1969. Granuloma formation induced in mice by chemically defined mycobacterial fractions. J. Bacteriol. 100:95–102.
117. **Berkerkunst, A., I. S. Levij, E. Yarkoni, E. Vilkas, and E. Lederer.** 1971. Suppression of urethan-induced lung adenomas in mice treated with trehalose-6,6′-dimycolate (cord factor) and living Bacillus Calmette Guérin. Science 174:1240–1242.
118. **Bekierkunst, A., L. Wang, R. Toubiana, and E. Lederer.** 1974. Immunotherapy of cancer with nonliving BCG and fractions derived from mycobacteria: role of cord factor (trehalose-6,6′-dimycolate) in tumor regression. Infect. Immun. 10:1044–1050.
119. **Bekierkunst, A., and E. Yarkoni.** 1973. Granulomatous hypersensitivity to trehalose-6,6′-dimycolate (cord factor) in mice infected with BCG. Infect. Immun. 7:631–638.
120. **Benacerraf, B., Z. Ovary, K. J. Bloch, and E. C. Franklin.** 1963. Properties of guinea pig 7S antibodies. I. Electrophoretic separation of two types of guines pig 7S antibodies. J. Exp. Med. 117:937–949.
121. **Ben-Efraim, S., M. Constantini-Sourojon, and D. W. Weiss.** 1973. Potentiation and modulation of the immune response of guinea pigs to poorly immunogenic protein-hapten conjugates by pretreatment with the MER fraction of attenuated tubercle bacilli. Cell. Immunol. 7:370–379.
122. **Berg, J. W.** 1953. Acid-fastness as a histochemical test. J. Histochem. Cytochem. 1:436–441.
123. **Berg, J. W.** 1953. Chemistry of acid-fastness. Proc. Soc. Exp. Biol. Med. 84:196–198.
124. **Berg, J. W.** 1953. The dual nature of acid-fastness. Yale J. Biol. Med. 26:215–223.
125. **Berg, J. W.** 1954. An acid-fast lipid from spermatozoa. Arch. Pathol. 57:115–120.
126. **Bergey, D. H., F. C. Harrison, R. S. Breed, B. W. Hammer, and F. M. Huntoon.** 1923. Bergey's manual of determinative bacteriology. 1st ed. Williams & Wilkins, Baltimore.
127. **Berken, A., and B. Benacerraf.** 1966. Properties of antibodies cytophilic for macrophages. J. Exp. Med. 123:119–144.
128. **Berset, C., and A. H. Etemadi.** 1973. Acyl-CoA-ACP-transacylases of *Mycobacterium smegmatis*. An analytical study. Biochimie 55:1381–1394.
129. **Berset, C., and A-H. Etemadi.** 1973. Séparation complète des activités palmityl-CoA-ACP-transacylasique et malonyl-CoA-ACP-transacylasique des homogénats de *Mycobacterium smegmatis*. C.R. Acad. Sci. Ser. D 297:1389–1392.
130. **Besse, P.** 1949. Epizootie à bacilles acido-résistants chez les poissons exotiques. Bull. Acad. Vet. Fr. 22:151–154.
130a. **Beytía, E. D., and J. W. Porter.** 1976. Biochemistry of polyisoprenoid biosynthesis. Annu. Rev. Biochem. 45;113–142.
131. **Bianco, C., R. Patrick, and V. Nussenzweig.** 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. Exp. Med. 132:702–720.
132. **Biberfeld, P., G. Biberfeld, P. Perlmann, and G. Holm.** 1973. Cytological observations on the cytotoxic interaction between lymphocytes and antibody-coated monolayer cells. Cell. Immunol. 7:60–72.
133. **Biggs, T. H.** 1971. Cervical lymphadenitis caused by atypical mycobacterium. J. K. Med. Assoc. 69:438–439.
134. **Birn, K. J., W. B. Schaefer, P. A. Jenkins, T. Szulga, and J. Marks.** 1967. Classification of *Mycobacterium avium* and related opportunist mycobacteria met in England and Wales. J. Hyg. 65:575–589.
135. **Birnbaum, S. E., and L. F. Affronti.** 1968. Chemical and serological relationships between the heteropolysaccharides of *Mycobacterium tuberculosis* and *Mycobacterium kansasii*. J. Bacteriol. 95:559–564.
136. **Birnbaum, S. E., and L. F. Affronti.** 1969. Mycobacterial polysaccharides. II. Comparison of polysaccharides from strains of four species of mycobacteria. J. Bacteriol. 100:58–63.
137. **Bishop, D. G., and E. Work.** 1965. An extracellular glycolipid produced by *Escherichia coli* grown under lysine-limiting conditions. Biochem. J. 96:567–576.
138. **Black, M. M., and W. L. Epstein.** 1974. Formation of multinucleate giant cells in organized epithelioid cell granulomas. Am. J. Pathol. 74:263–274.
139. **Back, W. A., and H. Tries.** 1972. Severe *Mycobacterium kansasii* infection of the lung. Can. J. Public Health 63:523–526.
140. **Blanden, R. V., M. J. Lefford, and G. B. Mackaness.** 1969. The host response to Calmette-Guérin bacillus infection in mice. J. Exp. Med. 129:1079–1101.
141. **Bloch, H.** 1950. Studies on the virulence of tubercle bacilli. Isolation and biological properties of a constituent of virulent orga-

nisms. J. Exp. Med. 91:197-218.
142. **Bloch, H., J. Defaye, E. Lederer, and H. Noll.** 1957. Constituents of a "toxic-lipid" obtained from *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 23:312-321.
143. **Bloch, K.** 1975. Fatty acids synthases from *Mycobacterium phlei*, p. 84-90. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 35. Lipids, part B. Academic Press Inc., New York.
144. **Bloch, K. J., F. M. Kourilsky, Z. Ovary, and B. Benacerraf.** 1963. Properties of guinea pig 7S antibodies. III. Identification of antibodies involved in complement fixation and hemolysis. J. Exp. Med. 117:965-981.
145. **Bloch-Shtacher, N., K. Hirschhorn, and J. W. Uhr.** 1968. The response of lymphocytes from non-immunized humans to antigen-antibody complexes. Clin. Exp. Immunol. 3:889-899.
146. **Bloom, B. R.** 1971. *In vitro* approaches to the mechanism of cell-mediated immune reactions. Adv. Immunol. 13:101-208.
147. **Bloom, B. R., and B. Bennett.** 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 153:80-82.
148. **Bluming, A. Z., C. L. Vogel, J. L. Ziegler, N. Mody, and G. Kamya.** 1972. Immunological effects of BCG in malignant melanoma. Two modes of administration compared. Ann. Intern. Med. 76:405-411.
149. **Boisvert, H.** 1965. *Mycobacterium xenopei* (Marks et Schwabacher 1965), mycobacterie scotochromogène, thermophile, dysgonique, éventuellement pathogène pour l'homme. Ann. Inst. Pasteur Paris 109:447-453.
150. **Bojalil, L. F.** 1959. Estudio comparativo entre *Mycobacterium marinum y Mycobacterium balnei*. Rev. Latinoam. Microbiol. 2:169-174.
151. **Bojalil, L. F., J. Cerbón, and A. Trujillo.** 1962. Adansonian classification of mycobacteria. J. Gen. Microbiol. 28:333-346.
152. **Bonhomme, F., C. Boucheron, D. Migliore, and P. Jollès.** 1966. Effet de l'acétylation sur les propiétés arthrogènes d'une fraction de cire D de *Mycobacterium tuberculosis* var. *hominis*. C.R. Acad. Sci. Ser. D 263: 1422-1425.
153. **Bönicke, R.** 1961. Die Bedeutung der Acylamidasen für die Identifizierung und Differenzierung der verschiedenen Arten der Gattung Mycobacterium. Jahresber. Borstel 5:7-87.
154. **Bönicke, R.** 1965. Beschreibung der neuen Species *Mycobacterium borstelense* n. sp. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. 196:535-538.
155. **Bönicke, R.** 1969. Lysogeny among mycobacteria. Folia Microbiol. (Prague) 14:297-304.
156. **Bönicke, R., and S. E. Juhasz.** 1964. Beschreibung der neuen Species *Mycobacterium vaccae* n. sp. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. 192:133-135.
157. **Bönicke, R., and S. E. Juhasz.** 1965. *Mycobakterium diernhoferi* n. sp., eine in der Umgebung des Rindes häufig vorkommende neue Mycobakterium-Species. Zentralbl. Bakteriol. Parasitenkd. Infektionsk. Hyg. Abt. I Orig. 197:292-294.
158. **Bonventre, P. E., and B. Black-Schaffer.** 1965. The effect of neutral and acidic polysaccharides on natural resistance of mice to bacterial challenge. J. Infect. Dis. 115:413-420.
159. **Bordet, C., M. Karahjoli, O, Gateau, and G. Michel.** 1972. Cell walls of nocardiae and related actinomycetes: identification of the genus *Nocardia* by cell wall analysis. Int. J. Syst. Bacteriol. 22:251-259.
160. **Borghans, J. G. A., and J. L. Stanford.** 1973. *Mycobacterium chelonei* in abscesses after injection of diphtheria-pertussis-tetanus-polio vaccine. Am. Rev. Respir. Dis. 107:1-8.
161. **Boros, D. L., and K. S. Warren.** 1973. The bentonite granuloma. Characterization of a model system for infectious and foreign body granulomatous inflammation using soluble mycobacterial, histoplasma and schistosoma antigens. Immunology 24:511-529.
162. **Bowles, J. A., and W. Segal.** 1965. Kinetics of utilization of organic compounds in the growth of *Mycobacterium tuberculosis*. J. Bacteriol. 90:157-163.
163. **Bowman, B. U., Jr.** 1958. Quantitative studies on some mycobacterial phage-host systems. J. Bacteriol. 76:52-62.
164. **Bowman, B. U.** 1968. Antigenicity of mycobacteriophages R1, D29 and Leo in rabbits. Proc. Soc. Exp. Biol. Med. 128:441-445.
165. **Bowman, B. U.** 1969. Effect of chloroform on mycobacteriophages. Microbios 1:347-352.
166. **Bowman, B. U.** 1969. Properties of mycobacteriophage DS6A. I. Immunogenicity in rabbits. Proc. Soc. Exp. Biol. Med. 131:196-200.
167. **Bowman, B. U., H. A. I. Newman, J. M. Moritz, and R. M. Koehler.** 1973. Properties of mycobacteriophage DS6A. II. Lipid composition. Am. Rev. Respir. Dis. 107:42-49.
168. **Bowman, B. U., Jr., and W. B. Redmond.** 1959. A temperate bacteriophage from *Mycobacterium butyricum*. Am. Rev. Respir. Dis. 80:232-239.
169. **Boyden, S. V.** 1964. Cytophilic antibody in guinea-pigs with delayed-type hypersensitivity. Immunology 7:474-483.
170. **Boyden, S. V., and E. Sorkin.** 1960. The adsorption of antigen by spleen cells previously treated with antiserum *in vitro*. Immunology 3:272-283.
171. **Boyden, S. V., and E. Sorkin.** 1961. The adsorption of antibody and antigen by spleen cells *in vitro*. Some further experiments. Immunology 4:244-252.

172. Boyer, H. W. 1971. DNA restriction and modification mechanisms in bacteria. Annu. Rev. Microbiol. 25:153-176.
173. Bradbury, F. C. S. 1946. Human pulmonary tuberculosis due to avian tubercle bacilli (pathological report by Young, J. A.). Lancet 250(1):89-91.
174. Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. 31:230-331.
175. Bradley, S. G. 1972. Reassociation of deoxyribonucleic acid from selected Mycobacteria with that from *Mycobacterium bovis* and *Mycobacterium farcinica*. Am. Rev. Respir. Dis. 106:122-124.
176. Bradley, S. G. 1973. Relationships among mycobacteria and nocardiae based upon deoxyribonucleic acid reassociation J. Bacteriol. 113:645-651.
177. Bradley, S. G., and J. S. Bond. 1974. Taxonomic criteria for mycobacteria and nocardiae. Adv. Appl. Microbiol. 18:131-190.
178. Brady, R. O. 1958. The enzymatic synthesis of fatty acids by aldol condensation. Proc. Natl. Acad. Sci. U.S.A. 44:993-998.
179. Brennan, P., and C. E. Ballou. 1967. Biosynthesis of mannophosphoinositides by *Mycobacterium phlei*. The family of dimannophosphoinositides. J. Biol. Chem. 242:3046-3056.
180. Brennan, P., and C. E. Ballou. 1968. Biosynthesis of mannophosphoinositides by *Mycobacterium phlei*. Enzymic acylation of the dimannophosphoinositides. J. Biol. Chem. 243:2975-2984.
181. Brennan, P. J., and D. P. Lehane. 1969. Acylglucoses in corynebacteria. Biochim. Biophys. Acta 176:675-677.
182. Brennan, P. J., and D. P. Lehane. 1971. The phospholipids of corynebacteria. Lipids 6:401-409.
183. Brennan, P. J., D. P. Lehane, and D. W. Thomas. 1970. Acylglucoses of the corynebacteria and mycobacteria. Eur. J. Biochem. 13:117-123.
184. Brennan, P. J., S. A. Rooney, and F. G. Winder. 1970. The lipids of *Mycobacterium tuberculosis* BCG: fractionation, composition, turnover and the effects of isoniazid. Ir. J. Med. Sci. 3:371-390.
185. Brieger, E. M., and H. B. Fell. 1945. Warm-stage observations on the initial development of the avian bacillus cultivated in embryo extract. J. Hyg. 44:158-169.
186. Brindle, T. W., M. I. Griffiths, T. Holme, K. Stalker, W. L. Burland, G. A. Coates, and P. W. Muggleton. 1972. A trial to compare reactions and responses to BCG vaccine prepared from Copenhagen 1331 and Glaxo 1077 strains. Tubercle 53:100-105.
187. Brindley, D. N., S. Matsumura, and K. Bloch. 1969. *Mycobacterium phlei* fatty acid synthetase; a bacterial multienzyme complex. Nature (London) 244:666-669.
188. British Medical Journal, Leading Articles. 1969. Of voles and men. Br. Med. J. 1:527-528.
189. Brody, J. A., T. Overfield, and L. M. Hammes. 1964. Depression of the tuberculin reaction by viral vaccines. N. Engl. J. Med. 271:1294-1296.
190. Brosbe, E. A., P. G. Fox, and J. H. Bates. 1970. Cytotoxic effect of *Mycobacterium tuberculosis* on J-111 cells. Tex. Rep. Biol. Med. 28:197-202.
191. Brown, C. A., P. Draper, and P. D. Hart. 1969. Mycobacteria and lysosomes: a paradox. Nature (London) 221:658-660.
192. Brown, K. A., and C. Ratledge. 1975. Iron transport in *Mycobacterium smegmatis:* ferrimycobactin reductase (NAD(P)H:ferrimycobactin oxidoreductase), the enzyme releasing iron from its carrier. FEBS Lett. 53:262-266.
193. Brownell, G. H., and J. N. Adams. 1967. Linkage and segregation of unselected markers in matings of *Nocardia erythropolis* and *Nocardia canicruria*. J. Bacteriol. 94:650-659.
194. Brundelet, P. J. 1973. Acid-fast staining of hooklets of *Taenia echinococcus*. Lancet i:678.
195. Bruneteau, M., and G. Michel. 1968. Structure d'un dimycolate d'arabinose isolé de *Mycobacterium marianum*. Chem. Phys. Lipids 2:229-239.
196. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins Co., Baltimore.
197. Buckle, G. 1972. Notes on *Mycobacterium ulcerans*. Aust. N. Z. J. Surg. 41:320-323.
198. Buhler, V. B., and A. Pollak. 1953. Human infection with atypical acid-fast organisms. Report of two cases with pathologic findings. Am. J. Clin. Pathol. 23:363-374.
199. Bullin, C. H., E. I. Tanner, and C. H. Collins. 1970. Isolation of *Mycobacterium xenopei* from water taps. J. Hyg. 68:97-100.
200. Buraczewska, M., B. Kwiatkowski, W. Manowski, and H. Rdultowska. 1972. Ultrastructure of some mycobacteriophages. Am. Rev. Respir. Dis. 105:22-29.
201. Buraczewska, M., W. Manowska, and H. Rdultowska. 1971. Induction of phages from lysogenic mycobacteria by means of ultraviolet irradiation. Am. Rev. Respir. Dis. 104:760-762.
202. Burchard, R. P., and M. Dworkin. 1966. Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. J. Bacteriol. 91:535-545.
203. Burchard, R. P., S. A. Gordon, and M. Dworkin. 1966. Action spectrum for the photolysis of *Myxococcus xanthus*. J. Bacteriol. 91:896-897.
204. Burchard, R. P., and S. B. Hendricks. 1969. Action spectrum for carotenogenesis in *Myxococcus xanthus*. J. Bacteriol. 97:1165-1168.
205. Burdon, K. L. 1946. Fatty material in bacte-

ria and fungi revealed by staining dried, fixed slide preparations. J. Bacteriol. 52:665–678.
206. **Burnet, F. M.** 1970. The concept of immunological surveillance. Prog. Exp. Tumor Res. 13:1–27.
207. **Burnet, F. M., J. F. McCrea, and J. D. Stone.** 1946. Modification of human red cells by virus action. I. The receptor gradient for virus action in human red cells. Br. J. Exp. Pathol. 27:228–236.
208. **Burnet, F. M., and J. D. Stone.** 1947. The receptor-destroying enzyme of *V. cholerae*. Aust. J. Exp. Biol. Med. Sci. 25:227–233.
209. **Cahall, D. L., and G. P. Youmans.** 1975. Conditions for production, and some characteristics, of mycobacterial growth inhibitory factor produced by spleen cells from mice immunized with viable cells of the attenuated H37Ra strain of *Mycobacterium tuberculosis*. Infect. Immun. 12:833–840.
210. **Cahall, D. L., and G. P. Youmans.** 1975. Molecular weight and other characteristics of mycobacterial growth inhibitory factor produced by spleen cells obtained from mice immunized with viable attenuated mycobacterial cells. Infect. Immun. 12:841–850.
211. **Cahall, D. L., and G. P. Youmans.** 1975. Macrophage migration inhibitory activity of mycobacterial growth inhibitory factor and the effect of a number of factors on mycobacterial growth inhibitory factor activity. Infect. Immun. 12:851–857.
212. **Cahn, B. J., and D. T. Smith.** 1972. Sporotrichoid *Mycobacterium marinum* infection: adjunct therapy with hyposensitization and x-ray. Cutis 9:485–490.
213. **Calmette, A., and C. Guérin.** 1908. Sur quelques propriétés du bacille tuberculeux cultivé sur la bile. C.R. Acad. Sci. 147:1456–1459.
214. **Canilang, B., and D. Armstrong.** 1968. Subcutaneous abscesses due to *Mycobacterium fortuitum*. Am. Rev. Respir. Dis. 97:451–454.
215. **Cann, D. C., and M. E. Willox.** 1964. Analysis of multimolecular enzymes as an aid to the identification of certain rapidly growing mycobacteria, using starch gel electrophoresis. J. Appl. Bacteriol. 28:165–173.
216. **Cantoni, G. L.** 1975. Biological methylation: selected aspects. Annu. Rev. Biochem. 44:435–451.
217. **Carr, I.** 1973. The macrophage, a review of ultrastructure and function, p. 18. Academic Press Inc., London.
218. **Casavant, C. H., and G. P. Youmans.** 1975. The adjuvant activity of mycobacterial RNA preparations and synthetic polynucleotides for induction of delayed hypersensitivity to purified protein derivative in guinea pigs. J. Immunol. 114:1014–1022.
219. **Casavant, C. H., and G. P. Youmans.** 1975. The induction of delayed hypersensitivity on guinea pigs to poly U and Poly A:U. J. Immunol. 114:1506–1509.
220. **Castelnuovo, G., G. Bellezza, and F. Borrelli.** 1970. Antigens of a mycobacteriophage: phlei phage. Ann. Inst. Pasteur (Paris) 118:214–219.
221. **Castelnuovo, G., G. Bellezza, and S. Yamanaka.** 1970. Phage inhibiting substances of Mycobacteria. I. The nature of phage inhibiting substances of *Mycobacterium phlei*. Ann. Inst. Pasteur Paris 119:302–311.
222. **Castelnuovo, G., A. Gaudiano, M. Morellini, and M. Polizzi-Sciarrone.** 1958. Differenziazione antigenica dei micobatteri studiata con le techniche di diffusione in agar, immunoelettroforesi e deviazione del complemento. Riv. Tuberc. Mal. Appar. Respir. 6:303–308.
223. **Castelnuovo, G., H. I. Giuliani, and E. L. de Giuliani.** 1966. Protein components of a mycobacteriophage. J. Gen. Virol. 4:253–257.
224. **Castelnuovo, G., S. Yamanaka, M. Zoppis, and E. Dotti.** 1969. The protein components of *Mycobacterium phlei:* fractionation procedures. Tubercle (London) 50:194–202.
225. **Castets, M., H. Boisvert, F. Grumbach, M. Brunel, and N. Rist.** 1968. Les bacilles tuberculeux de type Africain. Note preliminaire. Rev. Tuberc. Pneumol. 32:179–184.
226. **Castets, M., N. Rist, and H. Boisvert.** 1969. La variete africaine du bacille tuberculeux humain. Bull. Soc. Med. Afr. Noire Lang. Fr. 16:321–322.
227. **Cerottini, J.-C., and K. T. Brunner.** 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. Adv. Immunol. 18:67–132.
228. **Chamoiseau, G.** 1973. *Mycobacterium farcinogenes* agent causal du farcin du boeuf en Afrique. Ann. Microbiol. (Paris) 124A:215–222.
229. **Chamoiseau, G. and J. Asselineau.** 1970. Examen des lipides d'une souch de *Nocardia farcinica:* présence d'acides mycoliques. C. R. Acad. Sci. 270:2603–2604.
230. **Chang, C. S.** 1933. Human and bovine infection in extrapulmonary tuberculosis. N. Engl. J. Med. 209:690–692.
231. **Chang, Y. T., and R. N. Andersen.** 1969. Morphological changes of *Mycobacterium lepraemurium* grown in cultures of mouse peritoneal macrophages. J. Bacteriol. 99:867–875.
232. **Chaparas, S. D., and S. R. Hedrick.** 1973. Comparison of strains of BCG. I. Antigenic analysis and tuberculin reactivity. Infect. Immun. 7:777–780.
233. **Chaparas, S. D., D. E. Thor, and S. R. Hedrick.** 1971. Comparison of lymphocyte transformation, inhibition of macrophage migration and skin tests using dialyzable and nondialyzable tuberculin fractions from *Mycobacterium bovis* (BCG). J. Immunol. 107:149–153.
234. **Chapman, J. S. (ed.).** 1960. The anonymous

mycobacteria in human disease. Charles C Thomas, Springfield, Ill.
235. Chapman, J. S. 1967. Atypical mycobacterial infections. Pathogenesis, clinical manifestations and treatment. Med. Clin. North Am. 51:503-517.
236. Chapman, J. S., M. Dyerly, and S. Spohn. 1968. Epidemiological notes on *Mycobacterium kansasii* disease. Arch. Environ. Health 16:673-678.
237. Chaput, M., G. Michel, and E. Lederer. 1962. Structure du mycoside C_m, peptido-glycolipide de *Mycobacterium marianum*. Biochim. Biophys. Acta 63:310-326.
238. Chargaff, E. 1933. Sur les carotinoides des bactéries. C. R. Acad. Sci. 197:946-948.
239. Chargaff, E., and E. Lederer. 1935. Sur les pigments caroténoides de deux bactéries acido-résistantes. Ann. Inst. Pasteur Paris 54:383-388.
240. Chase, M. W., and H. Kowata. 1975. Multiple mycobacterial antigens in diagnostic tuberculins. Dev. Biol. Stand. 29:308-330.
241. Chaves-Carballo, E., and G. A. Sanchez. 1972. Regional lymphadenitis following BCG vaccination (BCGitis). Clinical comments based upon 25 instances among 1295 childhood vaccinees. Clin. Pediatr. (Philadelphia) 11:693-697.
242. Chavganc, J., R. Hanák, and H. G. Ten Dam. 1969. Epidemiological significance of the local reaction to direct BCG vaccination as assessed from a study in Mongolia. Bull. WHO 41:45-62.
243. Chedid, L., A. Lamensans, F. Parant, M. Parant, A. Adam, J.-F. Petit, and E. Lederer. 1973. Protective effect of delipidated mycobacterial cells and purified cell walls against Ehrlich carcinoma and a syngeneic lymphoid leukemia in mice. Cancer Res. 33:2187-2195.
244. Chester, F. D. 1901. A manual of determinative bacteriology. MacMillan Publishing Co., Inc., New York.
245. Cheung, O. T., and H. Konst. 1963. Pulmonary tuberculosis apparently caused by the avian tubercle bacillus. Can. Med. Assoc. J. 89:116-120.
246. Chipman, D. M., and N. Sharon. 1969. Mechanism of lysozyme action. Science 165:454-465.
247. Cianciulli, F. D. 1974. The radish bacillus (*Mycobacterium terrae*): saprophyte or pathogen? Am. Rev. Respir. Dis. 109:138-141.
248. Ciorbaru, R., A. Adam, J.-F. Petit, E. Lederer, C. Bona, and L. Chedid. 1975. Isolation of mitogenic and adjuvant active fractions from various species of *Nocardiae*. Infect. Immun. 11:257-264.
249. Claes, H., and T. O. M. Nakayama. 1959. Das photoxydative Ausbleichen von Chlorophyll *in vitro* in Gegewart von Carotinen mit verschiedenen chromophoren Gruppen. Z. Naturforsch. 14b:746-747.
250. Clancey, J. K. 1964. Mycobacterial skin ulcers in Uganda: description of a new mycobacterium (*Mycobacterium buruli*). J. Pathol. Bacteriol. 88:175-187.
251. Clancey, J. K., O. G. Dodge, H. F. Lunn, and M. L. Oduori. 1961. Mycobacterial skin ulcers in Uganda. Lancet 2:951-954.
252. Clapper, W. E., and J. Whitcomb. 1967. *Mycobacterium fortuitum* abscess at injection site. J. Am. Med. Assoc. 202:550.
253. Clasener, H. 1972. Pathogenicity of the L-phase of bacteria. Annu. Rev. Microbiol. 26:55-79.
254. Cleveland, D. E. H. 1951. Possible tuberculous skin infection from a swimming pool. Report of four cases. Acta Derm. Venereol. 31:147-152.
255. Collins, F. M. 1971. Immunogenicity of various mycobacteria and the corresponding levels of cross-protection developed between species. Infect. Immun. 4:688-696.
256. Comstock, G. W., L. B. Edwards, and H. Nabangxang. 1971. Tuberculin sensitivity eight to fifteen years after BCG vaccination. Am. Rev. Respir. Dis. 103:572-575.
257. Cone, R. E., and A. G. Johnson. 1971. Regulation of the immune system by synthetic polynucleotides. III. Action on antigen-reactive cells of thymic origin. J. Exp. Med. 133:665-676.
258. Cone, R. E., and J. D. Wilson. 1972. Adjuvant action of poly A:U on T and B rosette-forming cells in SRBC-immunized mice. Int. Arch. Allergy Appl. Immunol. 43:123-130.
259. Connor, D. H., and H. F. Lunn. 1965. *Mycobacterium ulcerans* infection (with comments on pathogenesis). Int. J. Lepr. 33: 698-705.
260. Connor, D. H., and H. F. Lunn. 1966. Buruli ulceration, a clinicopathologic study of 38 Ugandans with *Mycobacterium ulcerans* ulceration. Arch. Pathol. 81:183-199.
261. Convit, J., J. L. Avila, M. Goihman, and M. E. Pinardi. 1972. A test for the determination of competency in clearing bacilli in leprosy patients. Bull. WHO 46:821-826.
261a. Cooper, M. D., R. G. Keightley, and S. R. Webb. 1975. T- and B-cell interactions in autoimmune syndromes. Ann. N. Y. Acad. Sci. 256:105-116.
262. Corpe, R. F. 1964. Clinical aspects, medical and surgical, in the management of Battey-type pulmonary disease. Dis. Chest 45:380-382.
263. Corpe, R. F., E. S. Smith, and I. Stergus. 1961. Death due to *Mycobacterium fortuitum*. J. Am. Med. Assoc. 177:262-263.
264. Cortez, L. M., and G. A. Pankey. 1973. *Mycobacterium marinum* infections of the hand. Report of three cases and review of the literature. J. Bone Jt. Surg. 55A:363-370.
265. Coutinho, A., E. Gronowicz, W. W. Bullock, and G. Möller. 1974. Mechanism of thymus independent immunocyte triggering. Mitogenic activation of B cells results in specific

immune responses. J. Exp. Med. 139:73-92.
266. Coutinho, A., and G. Möller. 1973. B cell mitogenic properties of thymus-independent antigens. Nature (London) New Biol. 245:12-14.
266a. Coutinho, A., and G. Möller. 1975. Thymus-independent B-cell induction and paralysis. Adv. Immunol. 21:113-236.
267. Cozine, W. S., Jr., A. B. Stanfield, C. A. L. Stephens, Jr., and M. T. Mazur. 1973. Adjuvant disease-prevention with complete Freund's adjuvant and with mineral oil alone. Proc. Soc. Exp. Biol. Med. 143:528-530.
268. Crounse, J. B., R. P. Feldman, and R. K. Clayton. 1963. Accumulation of polyene precursors of neurosporene in mutant strains of *Rhodopseudomonas spheroides*. Nature (London) 198:1227-1228.
269. Crow, H. E., C. T. King, C. E. Smith, R. F. Corpe, and I. Stergus. 1957. A limited clinical, pathologic, and epidemiologic study of patients with pulmonary lesions associated with atypical acid-fast bacilli in the sputum. Am. Rev. Tuberc. Pulmon. Dis. 75:199-222.
270. Csillag, A. 1970. A simple method to obtain the mycococcus form of *Mycobacterium phlei*. J. Gen. Microbiol. 62:251-259.
271. Cummins, C. S. 1962. Chemical composition and antigenic structure of cell walls of *Corynebacterium, Mycobacterium, Nocardia, Actinomyces,* and *Arthrobacter*. J. Gen. Microbiol. 28:35-50.
272. Cummins, C. S. 1965. Chemical and antigenic studies on cell walls of mycobacteria, corynebacteria and nocardias. Am. Rev. Respir. Dis. 92:63-72.
273. Cummins, C. S., and H. Harris. 1958. Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. J. Gen. Microbiol. 18:173-189.
274. Cunto, G., F. Kanetsuna, and T. Imaeda. 1969. Chemical analysis of the mucopeptide of *Mycobacterium smegmatis*. Biochim. Biophys. Acta 192:358-360.
275. Cuttino, J. T., and A. M. McCabe. 1949. Pure granulomatous nocardiosis: a new fungus disease distinguished by intracellular parasitism. A description of a new disease in man due to a hitherto undescribed organism, *Nocardia intracellularis*, n. sp., including a study of the biologic and pathogenic properties of this species. Am. J. Pathol. 25:1-48.
276. da Costa Cruz, J. 1938. "*Mycobacterium fortuitum*." Um novo bacilo acido-resistente patogenico paro o homen. Acta Med. (Rio de Janeiro) 1:297-301.
277. Daniel, T. M. 1965. Observations of the antibody response of rabbits to mycobacterial antigens. J. Immunol. 95:100-108.
278. Daniel, T. M., and L. F. Affronti. 1973. Immunoelectrophoretic analysis of antigenic constituents of Seibert fractions of mycobacterial culture filtrates. Identification with the proposed United States-Japan reference nomenclature. Am. Rev. Resp. Dis. 108:1244-1248.
279. Daniel, T. M., and J. J. Wisnieski. 1970. The reaction of concanavalin-A with mycobacterial culture filtrates. Am. Rev. Resp. Dis. 101:762-764.
280. Dannenberg, A. M. 1975. Macrophages in inflammation and infection. N. Engl. J. Med. 293:489-493.
281. Dannenberg, A. M., Jr., M. Ando, and K. Shima. 1972. Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. III. The turnover of macrophages and its relation to their activation and antimicrobial immunity in primary BCG lesions and those of reinfection. J. Immunol. 109:1109-1121.
282. Dárziņš, E. 1950. Tuberculose des gias (*Leptodactylus pentadactylus*). Arq. IBIT (Inst. Bras. Invest. Tuberc.) 9:29-37.
283. Dárziņš, E., and G. Fahr. 1956. Cord-forming property, lethality and pathogenicity of mycobacteria. Dis. Chest 30:642-648.
284. Davey, M. E. 1967. Some clinical aspects and treatment of anonymous mycobacterial infections. Med. J. Aust. 2:438-441.
285. David, H. L. 1972. Response of *Mycobacterium tuberculosis* to environmental changes. Survival after transfer from amino acid-supplemented medium to basal medium. Am. Rev. Resp. Dis. 105:944-953.
286. David, H. L. 1973. Response of mycobacteria to ultraviolet light radiation. Am. Rev. Resp. Dis. 108:1175-1185.
287. David, H. L. 1974. Biogenesis of β-carotene in *Mycobacterium kansasii*. J. Bacteriol. 119:527-533.
288. David, H. L. 1974. Carotenoid pigments of *Mycobacterium kansasii*. Appl. Microbiol. 28:696-699.
289. David, H. L., D. S. Goldman, and K. Takayama. 1970. Inhibition of the synthesis of wax D peptidoglycolipid of *Mycobacterium tuberculosis* by D-cycloserine. Infect. Immun. 1:74-77.
290. David, J. R. 1973. Lymphocyte mediators and cellular hypersensitivity. N. Engl. J. Med. 288:143-149.
291. Davies, M., and D. E. S. Stewart-Tull. 1973. The dual affinity of a mycobacterial glycopeptide for sheep erythrocyte membranes and guinea-pig gamma-globulin. Immunology 25:1-9.
292. Davis, J. B., H. H. Chase, and R. L. Raymond. 1956. *Mycobacterium paraffinicum* n. sp., a bacterium isolated from soil. Appl. Microbiol. 4:310-315.
293. Davis, J. C., and S. Mudd. 1954. The cytology of a strain of *Corynebacterium diphtheriae*. J. Bacteriol. 69:372-386.
294. Davis, S. D., W. M. M. Kirby, and J. C. Sherris. 1966. Disseminated osteomyelitis due to

"Battey" mycobacteria. Am. Rev. Resp. Dis. 93:269–274.
295. Dennert, G., and D. Tucker. 1973. Antitumor polysaccharide lentinan-a T cell adjuvant. J. Natl. Cancer Inst. 51:1727–1729.
296. Desbordes, J., Savigny, J. Liaudet, G.-M. Lelieure, and G. Fouye. 1971. La xénopiose. Mycobactériose Havraise? Normande? Maritime? Arch. Med. Normand. 5:343–360.
297. DeWijs, H., and P. Jollés. 1964. Cell walls of three strains of mycobacteria (*Mycobacterium phlei, Mycobacterium fortuitum*, and *Mycobacterium kansasii*). Preparation, analysis and digestion by lysozymes of different origins. Biochim. Biophys. Acta 83:326–332.
298. Diara, A., and J. Pudles. 1959. Sur les lipides de *Corynebacterium ovis*. Bull. Soc. Chim. Biol. 41:481–486.
299. Dickey, R. F. 1968. Sporotrichoid mycobacteriosis caused by *M. marinum* (*balnei*). Arch. Dermatol. 98:385–391.
300. Dickler, H. B. and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. J. Exp. Med. 136:191–196.
301. Diena, B. B., R. Wallace, J. Carriere, and L. Greenberg. 1971. Observations on the antibody response to *Mycobacterium tuberculosis* in laboratory animals. Am. Rev. Resp. Dis. 104:757–759.
302. Dienes, L. 1928. Further observations concerning sensitization of tuberculous guinea pigs. J. Immunol. 15:153–174.
303. Dienes, L. 1928. The immunological significance of the tuberculous tissue. J. Immunol. 15:141–152.
304. Dienes, L. 1929. Technic of producing the tuberculin type of sensitization with egg white in tuberculous guinea pigs. J. Immunol. 17:531–538.
305. Dienes, L., and T. B. Mallory. 1937. The influence of allergy on the development of early tuberculous lesions. Am. J. Pathol. 13:897–910.
306. Dixon, J. M. S., and E. H. Cuthbert. 1967. Isolation of tubercle bacilli from uncentrifuged sputum on pyruvic medium. Am. Rev. Resp. Dis. 96:119–122.
307. Dodge, O. G. 1964. Mycobacterial skin ulcers in Uganda: histopathological and experimental aspects. J. Pathol. Bacteriol. 88:167–174.
308. Doke, S. 1959. Lysogenic strains and bacteriophages active against atypical mycobacteria. Med. Biol. (Tokyo) 53:253–256. (in Japanese)
309. Donald, K. J., and A. W. Pound. 1973. Proliferation and migration of reticuloendothelial cells following injection of a tubercle bacillary lipid. Br. J. Exp. Pathol. 54:79–89.
310. Donets, A. T., N. M. Gerasimova, V. V. Kotelev, and M. N. Bekhtereva. 1970. Dynamics of various fractions in the lipids during the development of *Mycobacterium rubrum* and *Mycobacterium album*. Microbiology 39: 675–677.
311. Doyle, W. M., L. C. Evander, and H. Gruft. 1968. Pulmonary disease caused by *Mycobacterium xenopei*. Am. Rev. Resp. Dis. 97:919–922.
312. Dragsted, J. 1949. Avian tuberculosis in man. Lancet 257(2):103–105.
313. Draper, P. 1974. The mycoside capsule of *Mycobacterium avium* 357. J. Gen. Microbiol. 83:431–433.
314. Draper, P., and R. J. W. Rees. 1970. Electron-transparent zone of mycobacteria may be a defense mechanism. Nature (London) 228:860–861.
315. Draper, P., and R. J. W. Rees. 1973. The nature of the electron-transparent zone that surrounds *Mycobacterium lepraemurium* inside host cells. J. Gen. Microbiol. 77:79–87.
316. Dresser, D. W. 1961. Effectiveness of lipid and lipidophilic substances as adjuvants. Nature (London) 191:1169–1171.
317. Dresser, D. W. 1972. The role of T cells and adjuvant in the immune response of mice to foreign erythrocytes. Eur. J. Immunol. 2:50–57.
318. Dresser, D. W. (ed.) 1976. Immunological tolerance. Br. Med. Bull. 32:99–185.
319. Dresser, D. W., and J. M. Phillips. 1973. The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants, p. 3–28. *In* G. E. W. Wolstenholme and J. Knight (ed.). Immunopotentiation. Ciba Found. Symp. 18 (new ser.). Associated Scientific Publishers, Amsterdam.
319a. Dresser, D. W., and A. M. Popham. 1976. Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide. Nature (London) 264:552–554.
320. Dross, I. C., A. A. Abbatiello, F. S. Jenney, and A. C. Cohen. 1964. Pulmonary infection due to *M. fortuitum*. Am. J. Resp. Dis. 89:923–925.
321. Dubos, R. J. 1947. The effect of lipids and serum albumin on bacterial growth. J. Exp. Med. 85:9–22.
322. Dubos, R. J., and G. Middlebrook. 1947. Media for tubercle bacilli. Am. Rev. Tuberc. 56:334–345.
323. Dukor, P., and K. U. Hartmann. 1973. Bound C3 as the second signal for B-cell activation. Cell. Immunol. 7:349–356.
324. Dumonde, D. C., R. A. Wolstencroft, G. S. Panayi, M. Matthew, J. Morley, and W. T. Howson. 1969. "Lymphokines": nonantibody mediators of cellular immunity generated by lymphocyte activation. Nature (London) 224:38–42.
325. Dumont, A., and H. Sheldon. 1965. Changes in the fine structure of macrophages in experimentally produced tuberculous granulomas in hamsters. Lab. Invest. 14:2034–2055.
326. Dunsford, H. A., H. L. Lucia, B. L. Doughty,

and F. von Lichtenberg. 1974. Artificial granulomas from bentonite and latex carrier particles. Am. J. Trop. Med. Hyg. 23:203-217.
327. Dutton, R. W., R. Falkoff, J. A. Hirst, M. Hoffmann, J. W. Kappler, J. R. Kettman, J. F. Lesley, and D. Vann. 1971. Is there evidence for a non-antigen specific diffusable chemical mediator from the thymus-derived cell in the initiation of the immune response? p. 355-368. *In* B. Amos (ed.), Progress in immunology. 1st International Congress of Immunology. Academic Press Inc., New York.
328. Duval, C. W. 1910. The cultivation of the leprosy bacillus and the experimental production of leprosy in the Japanese dancing mouse. J. Exp. Med. 12:649-665.
329. Dvorak, H. F., R. B. Colvin, and W. H. Churchill. 1975. Specificity of basophils and lymphocytes in cutaneous basophil hypersensitivity. J. Immunol. 114:507-511.
330. Dvorak, H. F., B. A. Simpson, R. C. Bast, Jr., and S. Leskowitz. 1971. Cutaneous basophil hypersensitivity. III. Participation of the basophil in hypersensitivity to antigen-antibody complexes, delayed hypersensitivity and contact allergy. Passive transfer. J. Immunol. 107:138-148.
331. Dworkin, M. 1958. Endogenous photosensitization in a carotenoidless mutant of *Rhodopseudomonas spheroides*. J. Gen. Physiol. 41:1099-1112.
332. Edmond, E., A. J. Keay, and A. Wallace. 1968. Avian tuberculosis. Scott. Med. J. 13:379-381.
333. Edwards, L. B. 1963. Current status of the tuberculin test. Ann. N. Y. Acad. Sci. 106:32-42.
334. Edwards, L. B., L. Hopwood, and C. E. Palmer. 1965. Identification of mycobacterial infections. Bull. WHO 33:405-412.
335. Eisen, H. N. 1974. Immunology: an introduction to molecular and cellular principles of the immune responses, 2nd ed. Harper and Row, Publishers, Hagerstown, Md.
336. Eisenstein, T. K. 1975. Evidence for O antigens as the antigenic determinants in "ribosomal" vaccines prepared from *Salmonella*. Infect. Immun. 12:364-377.
337. Eisler, D. M., and R. J. Heckly. 1968. Possible mechanisms of action of an anti-*Pasteurella pestis* factor. J. Bacteriol. 96:1977-1981.
338. Elberg, S. S., P. Schneider, and J. Fong. 1957. Cross-immunity between *Brucella melitensis* and *Mycobacterium tuberculosis*. Intracellular behavior of *Brucella melitensis* in monocytes from vaccinated animals. J. Exp. Med. 106:545-554.
339. Elek, G., E. Vandra, and I. Foldes. 1969. Production of antibodies against *Mycobacterium phlei* phage. Acta Microbiol. Acad. Sci. Hung. 16:69-75.
340. Elliott, B. E., and J. S. Haskill. 1974. Separation of T effector cells in humoral and cellular immunity. Nature (London) 252:607-608.
341. Elliott, R. C., W. H. Spaur, and J. W. Sokolowski. 1969. The incidence of tuberculosis among Marine Corps and naval personnel in Vietnam. Transactions of the 28th VA-AF Pulmonary Disease Research Conference, p. 6.
342. Ellouz, F., A. Adam, R. Ciorbuar, and E. Lederer. 1974. Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochem. Biophys. Res. Commun. 59:1317-1325.
343. Elston, H. R., and J. P. Duffy. 1973. *Mycobacterium xenopi* and mycobacteriosis. Am. Rev. Resp. Dis. 108:944-949.
344. Elting, J. J., and W. O. Southwick. 1974. Acute infantile septic arthritis of the hip due to *Mycobacterium triviale*. J. Bone Jt. Surg. 56A:184-186.
345. Engbaek, H. C. 1964. Lymph gland processes caused by atypical mycobacteria and *M. avium*. Acta Tuberc. Scand. 44:108-137.
346. Engbaek, H. C. 1964. Three cases in the same family of fatal infection with *M. avium*. Acta Tuberc. Scand. 45:105-117.
347. Engbaek, H. C., B. Vergmann, I. Baess, and D. W. Will. 1967. *M. xenopei*. A bacteriological study of *M. xenopei* including case reports of Danish patients. Acta Pathol. Microbiol. Scand. 69:576-594.
348. Engstrom, P. F., G. C. Dewey, and O. Barrett, Jr. 1972. Disseminated *Mycobacterium kansasii* infection. Successful treatment of a patient with pancytopenia. Am. J. Med. 52:533-537.
349. Epstein, W. L. 1967. Granulomatous hypersensitivity. Prog. Allergy 11:36-88.
350. Erfle, J. D. 1973. Acetyl-CoA and propionyl-CoA carboxylation by *Mycobacterium phlei*. Partial purification and some properties of the enzyme. Biochim. Biophys. Acta 316:143-155.
351. Espejo, R. T., and E. S. Canelo. 1968. Properties of bacteriophage PM2: a lipid containing bacterial virus. Virology 34:738-747.
352. Etémadi, A.-H. 1965. Sur l'intérêt taxinomique et la signification phylogénétique des acides mycoliques. Mem. Soc. Bot. Fr. p. 47-71.
353. Etémadi, A.-H. 1967. Corrélations structurales et biogénétiques des acides mycoliques en rapport avec la phylogenèse de quelques generes d'Actinomycétales. Bull. Soc. Chim. Biol. 49:695-706.
354. Etémadi, A.-H. 1967. Les acides mycoliques structure, biogenèse et intérêt phylogénétique. Exp. Annu. Biochim. Med. 28, p. 77-109.
355. Etémadi, A.-H., and J. Gasche. 1967. Sur l'origine biogénétique du 2-eicosanol et du 2-octadécanol de *Mycobacterium avium*. Bull. Soc. Chim. Biol. 47:2095-2104.
356. Etémadi, A.-H., and E. Lederer. 1965. Biosynthèse de l'acide α-smegma-mycolique. Biochim. Biophys. Acta 98:160-167.

357. Etémadi, A.-H., R. Okuda, and E. Lederer. 1964. Sur la structure de l'acide α-smegmamycolique. Bull. Soc. Chim. Fr., p. 868–870.
358. Evans, R., and P. Alexander. 1971. Rendering macrophages specifically cytotoxic by a factor released from immune lymphoid cells. Transplantation 12:227–229.
359. Evans, R., and P. Alexander. 1972. Mechanism of immunologically specific killing of tumour cells by macrophages. Nature (London) 236:168–170.
359a. Fagiolo, E. 1976. Immunochemical study of incomplete platelet autoantibodies by the anti-globulin consumption test (AGCT) with specific anti-immunoglobulin sera. J. Immunol. Methods 9:225–229.
360. Falk, G. A., S. J. Hadley, F. E. Sharkey, M. Liss, and C. Muschenheim. 1973. *Mycobacterium avium* infections in man. Am. J. Med. 54:801–810.
361. Farber, E. R., and A. Tsang. 1967. Mycobacterial ("Buruli") ulcer in a Peace Corps worker. Arch. Surg. (Chicago) 95:297–300.
362. Fay, D. D., and B. U. Bowman. 1971. Characterization of mycobacteriophage R1. Bacteriol. Proc. 1971:174.
363. Feinberg, K. A., and S. S. Schneierson. 1969. Disseminated infection by *Mycobacterium fortuitum*. Mt. Sinai J. Med. N. Y. 36:375–379.
364. Feit, C., and R. P. Tewari. 1974. Immunogenicity of ribosomal preparations from yeast cells of *Histoplasma capsulatum*. Infect. Immun. 10:1091–1097.
365. Feldman, R. A., and E. Hershfield. 1974. Mycobacterial skin infection by an unidentified species. A report of 29 patients. Ann. Intern. Med. 80:445–452.
366. Feldman, W. H. 1938. Avian tuberculosis infections. The Williams & Wilkins Co., Baltimore.
367. Feldman, W. H. 1947. Animal tuberculosis and its relationship to the disease in man. Ann. N. Y. Acad. Sci. 48:469–505.
368. Feldman, W. H., and R. E. Ritts. 1963. Pathogenicity studies of group III (Battey) mycobacteria from pulmonary lesions of man. Dis. Chest 43:26–33.
368a. Feldmann, M. 1972. Cell interactions in the immune response in vitro. V. Specific collaboration via complexes of antigen and thymus-derived cell immunoglobulin. J. Exp. Med. 136:737–760.
368b. Feldmann, M. 1973. Induction of B cell tolerance by antigen specific T cell factor. Nature (London) New Biol. 242:82–84.
369. Feldmann, M., and A. Basten. 1971. The relationship between antigenic structure and the requirement for thymus-derived cells in the immune response. J. Exp. Med. 134:103–119.
370. Fenner, F. 1950. The significance of the incubation period in infectious diseases. (The original designation, *M. ulcerans*, is credited to MacCallum, Tolkurst and Buckle, herein). Med. J. Aust. 2:813–818.
371. Ferguson, J. A., and C. E. Ballou. 1970. Biosynthesis of a mycobacterial lipopolysaccharide. Properties of the polysaccharide methyltransferase. J. Biol. Chem. 245: 4213–4223.
372. Fields, B. T., Jr., M. C. Bishop, E. A. Brosbe, and J. H. Bates. 1969. Pulmonary disease caused by *Mycobacterium xenopei* and *Histoplasma capsulatum*: a case report. Am. Rev. Resp. Dis. 99:590–594.
373. Fife, E. H., Jr., R. H. Kruse, A. J. Toussaint, and E. V. Staab. 1970. Serodiagnosis of simian tuberculosis by soluble antigen fluorescent antibody (SAFA) tests. Lab. Anim. Care 20:969–978.
374. Fincher, G. B., W. H. Sawyer, and B. A. Stone. 1974. Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. Biochem. J. 139:535–545.
375. Fischel, E. E., E. A. Kabat, H. C. Stoerk, and A. E. Bezer. 1952. The role of tubercle bacilli in adjuvant emulsions on antibody production to egg albumin. J. Immunol. 69:611–618.
376. Fischer, D. A., W. Lester, and W. B. Schaefer. 1968. Infections with atypical mycobacteria. Five years' experience at the National Jewish Hospital. Am. Rev. Resp. Dis. 98:29–34.
377. Fisher, C. A., and L. Barksdale. 1973. Cytochemical reactions of human leprosy bacilli and mycobacteria: ultrastructural implications. J. Bacteriol. 113:1389–1399.
378. Fisher, D. A., and R. S. Lyss. 1974. *Mycobacterium marinum* infection. Arch. Dermatol. 109:571.
379. Fiume, L., and T. Wieland. 1970. Amanitins. Chemistry and action. FEBS Lett. 8:1–5.
380. Fleer, M. A., and B. U. Bowman. 1973. Partial characterization of mycobacteriophage R1 particles surviving chloroform treatment. Microbios 7:37–44.
381. Flick, P. K., and K. Bloch. 1974. *In vitro* alterations of the product distribution of the fatty acid synthetase from *Mycobacterium phlei*. J. Biol. Chem. 249:1031–1036.
381a. Flower, R. L. P., K. J. Turner, and M. P. Alpers. 1975. Mechanism of an action of an antibacterial murine lymphokine. Nature (London) 254:459–460.
382. Flowers, D. J. 1970. Human infection due to *Mycobacterium marinum* after a dolphin bite. J. Clin. Pathol. 20:475–477.
383. Fogan, L. 1970. Atypical mycobacteria. Their clinical, laboratory, and epidemiological significance. Medicine 49:243–255.
384. Földes, I., and P. Somogyi. 1968. Basenanalyse der Nukleinsäuren von Mykobakterien. Z. Tuberk. 128:151–155.
385. Fong, J., D. Chin, H. J. Akiyama, and S. S. Elberg. 1959. Studies on tubercle bacillus-monocyte relationship. III. Conditions affecting the action of serum and cells. Modifications of bacilli in an immune system. J.

Exp. Med. 109:523-543.
386. **Fong, J., P. Schneider, and S. S. Elberg.** 1957. Studies on tubercle bacillus-monocyte relationship. II. Induction of monocyte degeneration by bacteria and culture filtrate: specificity of serum and monocyte effects on resistance to degeneration. J. Exp. Med. 105:25-37.
387. **Foote, C. S.** 1968. Mechanisms of photosensitized oxidation. Science 162:963-970.
388. **Forney, F. W., and A. J. Markovetz.** 1968. Oxidative degradation of methyl ketones. II. Chemical pathway for degradation of 2-tridecanone by *Pseudomonas aeruginosa*. J. Bacteriol. 96:1055-1064.
389. **Forsgren, A.** 1970. Significance of protein A production by staphylococci. Infect. Immun. 2:672-673.
390. **Forsgren, A., and J. Sjöquist.** 1966. "Protein A" from *S. aureus*. I. Pseudo-immune reaction with human γ-globulin. J. Immunol. 97:822-827.
391. **Fox, T. G.** 1972. Non-tuberculous mycobacterial infections. N. Z. Med. J. 76:93-97.
392. **Francis, J.** 1943. Infection of laboratory animals with *Mycobacterium johnei*. J. Comp. Pathol. Ther. 53:140-150.
393. **Freedman, S. O., R. Turcotte, A. J. Fish, and A. H. Sehon.** 1963. The *in vitro* detection of "cell-fixed" hemagglutinating antibodies to tuberculin purified protein derivative (PPD) in humans. J. Immunol. 90:52-59.
394. **Freer, J., K. S. Kim, M. R. Krauss, L. Beaman, and L. Barksdale.** 1969. Ultrastructural changes in bacteria isolated from cases of leprosy. J. Bacteriol. 100:1062-1075.
395. **Fregnan, G. B., and D. W. Smith.** 1962. Description of various colony forms of mycobacteria. J. Bacteriol. 83:819-827.
396. **Fregnan, G. B., D. W. Smith, and H. M. Randall.** 1962. A mutant of a scotochromogenic *Mycobacterium* detected by colony morphology and lipid studies. J. Bacteriol. 83:828-836.
397. **Frenkel, J. K.** 1967. Adoptive immunity to intracellular infection. J. Immunol. 98:1309-1319.
398. **Freund, J.** 1956. The mode of action of immunologic adjuvants. Adv. Tuberc. Res. 7:130-148.
399. **Freund, J., and M. M. Lipton.** 1948. Potentiating effect of *Nocardia asteroides* on sensitization to picryl chloride and on production of isoallergic encephalomyelitis. Proc. Soc. Exp. Biol. Med. 68:373-377.
400. **Freund, J., and S. H. Stone.** 1959. The effectiveness of tuberculo-glycolipid as an adjuvant in eliciting allergic encephalomyelitis and aspermatogenesis. J. Immunol. 82:560-567.
401. **Friedberg, D., and M. Shilo.** 1965. Infection-promoting activity of high molecular weight microbial polysaccharides on mouse peritoneal infections with *Pasteurella pestis*. J. Infect. Dis. 115:263-270.
402. **Friedman, H. M., A. G. Johnson, and P. Pan.** 1969. Stimulatory effect of polynucleotides on short term leukocyte cultures. Proc. Soc. Exp. Biol. Med. 132:916-918.
403. **Friedmann, F. F.** 1903. Spontane Lungentuberkulose bei Schildkröten und die Stellung des Tuberkelbazillus im System. Mit einer Übersicht über die Lehre von der Kaltblütertuberkulose. Z. Tuberk. Erkr. Thoraxorgane 4:439-457.
404. **Froman, S., R. Burge, M. Gedebou, and M. J. Pickett.** 1968. Serologic testing for tuberculosis. Am. Rev. Resp. Dis. 97:201-205.
405. **Froman, S., M. D. Lechtman, L. Scammon, D. W. Will, and B. R. Eckmann.** 1961. Mycobacteriophage lysates as serologic antigens. Am. Rev. Resp. Dis. 83:901-902.
406. **Froman, S., and L. Scammon.** 1964. Effect of temperature on the bacteriophage susceptibility of strains of *Mycobacterium avium* isolated from fowl. Am. Rev. Resp. Dis. 89:236-239.
407. **Froman, S., D. W. Will, and E. Bogen.** 1954. Bacteriophage active against virulent *Mycobacterium tuberculosis*. I. Isolation and activity. Am. J. Public Health 44:1326-1333.
408. **Frost, P., and E. M. Lance.** 1973. The relation of lymphocyte trapping to the mode of action of adjuvants, p. 29-45. *In* G. E. W. Wolstenholme and J. Knight (ed.), Immunopotentiation. Ciba Found. Symp. no. 18 (new ser.). Associated Scientific Publishers, Amsterdam.
409. **Frouin, A.** 1921. Sur la teneur en matières grasses des bacilles tuberculeux des types humain, bovin, aviaire. C. R. Soc. Biol. 84:606-608.
410. **Fulco, A. J.** 1974. Metabolic alterations of fatty acids. Annu. Rev. Biochem. 43:215-241.
411. **Furth, J.** 1926. On the serological relationship of acid-fast bacilli. J. Immunol. 12:273-292.
412. **Furuchi, A., and T. Tokunaga.** 1972. Nature of the receptor substance of *Mycobacterium smegmatis* for D4 bacteriophage adsorption. J. Bacteriol. 111:404-411.
413. **Gaisford, W.** 1955. The protection of infants against tuberculosis. Br. Med. J. 2:1164-1171.
414. **Galindo, B.** 1972. Antigen-mediated fusion of specifically sensitized rabbit alveolar macrophages. Infect. Immun. 5:583-594.
415. **Galindo, B., J. Lazdins, and R. Castillo.** 1974. Fusion of normal rabbit alveolar macrophages induced by supernatant fluids from BCG-sensitized lymph node cells after elicitation by antigen. Infect. Immun. 9:212-216.
416. **Galindo, B., and Q. N. Myrvik.** 1970. Migratory response of granulomatous alveolar cells from BCG-sensitized rabbits. J. Immunol. 105:227-237.
417. **Galli-Valerio, B.** 1912. Études sur les actinomycètes. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 63:555-564.

418. Galli-Valerio, B., and M. Bornand. 1927. Le *Mycobacterium aquae* Galli-Valerio et son action pathogene. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 101:182-193.
419. Gangadharam, P. R., and K. H. K. Hsu. 1972. *Mycobacterium abcessus* infection in a puncture wound. Am. Rev. Resp. Dis. 106:275-277.
420. Gastambide-Odier, M. 1973. Variantes de mycosides caractérisée par des résidus glycosidiques substitués par des chaînes acyles. Nature mycolique des mycosides G'. Eur. J. Biochem. 33:81-86.
421. Gastambide-Odier, M., and E. Lederer. 1959. Biosynthesis of corynemycolic acid from two molecules of palmitic acid. Nature (London) 184:1563-1564.
422. Gastambide-Odier, M., and E. Lederer. 1960. Biosynthèse de l'acide corynemycolique à partir de deux molécules d'acide palmatique. Biochem. Z. 333:285-295.
423. Gastambide-Odier, M., and P. Sarda. 1970. Contribution â l'acide de la structure et de la biosynthèse de glycolipides spécifiques isolés de mycobactéries: les mycosides A et B. Pneumonologie 142:241-255.
424. Gastambide-Odier, M., and C. Ville. 1970. Desoxysucres isolés du mycoside A: identification des dérivés acétylés des méthyl 2,4-di-O-methyl-rhamnopyranoside, 2-O-méthyl-rhamnofuranoside, 3-O-méthyl-rhamnofuranoside, 2-O-méthyl-fucopyranoside et 3-O-méthyl-fucofuranoside. Bull. Soc. Chim. Biol. 52:679-693.
425. Gelbart, S. M., and S. E. Juhasz. 1970. Genetic transfer in *Mycobacterium phlei*. J. Gen. Microbiol. 64:253-254.
426. Gelbart, S. M., and S. E. Juhasz. 1973. Transduction in *Myobacterium phlei*. Antonie van Leeuwenhoek; J. Microbiol. Serol. 39:1-10.
427. George, M., and J. H. Vaughan. 1962. *In vitro* cell migration as a model for delayed hypersensitivity. Proc. Soc. Exp. Biol. Med. 111:514-521.
427a. Germain, R. N., R. M. Williams, and B. Benacerraf. 1975. Specific and nonspecific antitumor immunity. II. Macrophage-mediated nonspecific effector activity induced by BCG and similar agents. J. Natl. Cancer Inst. 54:709-720.
428. Gershon, R. K. 1974. Lack of activity of contra-suppressor T cells as a mechanism of tolerance, p. 441-469. *In* D. H. Katz and B. Benacerraf (ed.), Immunological tolerance; mechanisms and potential therapeutic applications. Academic Press Inc., New York.
429. Gershon, R. K. 1974. T cell control of antibody production, p. 1-40. *In* M. D. Cooper and N. L. Warner (ed.), Contemporary topics in immunobiology, vol. 3. Plenum Press, New York.
430. Ghuysen, J.-M. 1968. Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. Bacteriol. Rev. 32:425-464.
431. Girard, D. E., G. C. Bagby, Jr., and J. R. Walsh. 1973. Destructive polyarthritis secondary to *Mycobacterium kansasii*. Arthritis Rheum. 16:665-669.
432. Glenchur, H., R. E. Burge, and S. Froman. 1973. Tuberculin activity of mycobacterial fractions obtained by chromatography. Appl. Microbiol. 25:5-10.
433. Glynn, L. E. 1968. The chronicity of inflammation and its significance in rheumatoid arthritis. Ann. Rheum. Dis. 27:105-121.
434. Godwin, M. C. 1965. Infection of knee joint by *Mycobacterium kansasii*. J. Am. Med. Assoc. 194:88-89.
435. Goldman, D. S. 1961. Enzyme systems in mycobacteria. A review. Adv. Tuberc. Res. 11:1-44.
436. Goldman, D. S. 1970. Subcellular localization of individual mannose-containing phospholipids in *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 102:543-555.
437. Goldstein, A. L., A. Guha, M. L. Howe, and A. White. 1971. Ontogenesis of cell-mediated immunity in murine thymocytes and spleen cells and its acceleration by thymosin, a thymic hormone. J. Immunol. 106:773-780.
438. Goldstein, I. J., and A. Misaki. 1970. Interaction of concanavalin A with an arabinogalactan from the cell wall of *Mycobacterium bovis*. J. Bacteriol. 103:422-425.
439. Gomes, N. F., T. Ioneda, and J. Pudles. 1966. Purification and chemical constitution of the phospholipids from *Corynebacterium diphtheriae* P.W. 8. Nature (London) 211:81-82.
440. Goodfellow, M. 1971. Numerical taxonomy of some nocardioform bacteria. J. Gen. Microbiol. 69:33-80.
441. Goodfellow, M., D. E. Minnikin, P. V. Patel, and H. Mordarska. 1973. Free nocardomycolic acids in the classification of nocardias and strains of the "rhodochrous" complex. J. Gen. Microbiol. 74:185-188.
442. Goodwin, T. W. 1959. The biosynthesis and function of the carotenoid pigments. Adv. Enzymol. 21:295-368.
443. Goodwin, T. W. 1971. Biosynthesis, p. 577-636. *In* O. Isler (ed.), Carotenoids. Birkhäuser, Basel.
444. Goodwin, T. W. 1971. Biosynthesis of carotenoids and plant triterpenes. Biochem. J. 123:293-329.
445. Gordon, J., and R. G. White. 1971. Surface peptido-glycolipid filaments on *Mycobacterium leprae*. Clin. Exp. Immunol. 9:539-547.
446. Gordon, R. E. 1966. Some strains in search of a genus—*Corynebacterium, Mycobacterium, Nocardia* or what? J. Gen. Microbiol. 43:329-343.
447. Gordon, R. E., and J. M. Mihn. 1959. A comparison of four species of mycobacteria. J. Gen. Microbiol. 21:736-748.
448. Gordon, R. E., and M. M. Smith. 1953. Rap-

idly growing, acid fast bacteria. I. Species' descriptions of *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. J. Bacteriol. 66:41–48.

449. **Gordon, R. E., and M. M. Smith.** 1955. Rapidly growing, acid fast bacteria. II. Species description of *Mycobacterium fortuitum* Cruz. J. Bacteriol. 69:502–507.
450. **Goren, M. B.** 1970. Sulfolipid I of *Mycobacterium tuberculosis*, strain $H_{37}Rv$. I. Purification and properties. Biochim. Biophys. Acta 210:116–126.
451. **Goren, M. B.** 1970. Sulfolipid I of *Mycobacterium tuberculosis*, strain $H_{37}Rv$. II. Structural studies. Biochim. Biophys. Acta 210:127–138.
452. **Goren, M. B.** 1971. Mycobacterial sulfolipids: spontaneous desulfation. Lipids 6:40–46.
453. **Goren, M. B.** 1972. Mycobacterial lipids: selected topics. Bacteriol. Rev. 36:33–64.
454. **Goren, M. B.** 1975. Cord factor revisited: a tribute to the late Dr. Hubert Bloch. Tubercle 56:65–71.
455. **Goren, M. B., and O. Brokl.** 1974. Separation and purification of cord factor (6,6′ dimycoloyl trehalose) from wax C or from mycolic acids. Recent Results Cancer Res. 47:251–258.
456. **Goren, M. B., O. Brokl, B. C. Das, and E. Lederer.** 1971. Sulfolipid I of *Mycobacterium tuberculosis*, strain H37Rv. Nature of the acyl substituents. Biochemistry 10:72–81.
457. **Goren, M. B., O. Brokl, and W. B. Schaefer.** 1974. Lipids of putative relevance to virulence in *Mycobacterium tuberculosis*: correlation of virulence with elaboration of sulfatides and strongly acidic lipids. Infect. Immun. 9:142–149.
458. **Goren, M. B., O. Brokl, and W. B. Schaefer.** 1974. Lipids of putative relevance to virulence in *Mycobacterium tuberculosis*: phthiocerol dimycocerosate and the attenuation indicator lipid. Infect. Immun. 9:150–158.
459. **Goren, M. B., J. K. McClatchy, B. Martens, and O. Brokl.** 1972. Mycosides C: behavior as receptor site substance for mycobacteriophage D4. J. Virol. 9:999–1003.
460. **Gosling, R., and J. Montanus.** 1924. Direct cultivation of tubercle bacilli from spinal fluids. Occurrence of human and bovine types. J. Med. Res. 44:513–519.
461. **Gould, W. M., D. R. McMeekin, and R. D. Bright.** 1968. *Mycobacterium marinum* (*balnei*) infection. Report of a case with cutaneous and laryngeal lesions. Arch. Dermatol. 97:159–162.

461a. **Grabar, P.** 1975. Hypothesis. Auto-antibodies and immunological theories: an analytical review. Clin. Immunol. Immunopathol. 4:453–466.

462. **Grange, J. M., and G. Nordstrom.** 1973. Bacteriophage typing of *Mycobacterium ranae* (*fortuitum*). The correlation of lysis by mycobacteriophage BK4 and inositol utilisation. Acta Pathol. Microbiol. Scand. B. 81:408–412.
463. **Granger, D. L., K.-I. Yamamoto, and E. Ribi.** 1976. Delayed hypersensitivity and granulomatous response after immunization with protein antigens associated with a mycobacterial glycolipid and oil droplets. J. Immunol. 116:482–488.
464. **Grant, J.** 1971. Selective mycobacteriophage isolation with membrane filters. Appl. Microbiol. 21:1091.
465. **Gratia, A.** 1937. Le phénomène du halo et la synergie des bacteriophages. C.R. Soc. Biol. 126:418–421.
466. **Gray, D. F.** 1953. Detection of small numbers of mycobacteria in sections by fluorescence microscopy. Am. Rev. Tuberc. 68:82–95.
467. **Gray, G. R., and C. E. Ballou.** 1972. The 6-O methylglucose-containing lipopolysaccharides of *Mycobacterium phlei*. Locations of the acyl groups. J. Biol. Chem. 247:8129–8135.
468. **Graybill, J. R., J. Silva, Jr., D. W. Fraser, R. Lordon, and E. Rogers.** 1974. Disseminated mycobacterioses due to *Mycobacterium abscessus* in two recipients of renal homografts. Am. Rev. Respir. Dis. 109:4–10.
469. **Greenawalt, J. W., and T. L. Whiteside.** 1975. Mesosomes: membranous bacterial organelles. Bacteriol. Rev. 39:405–463.

469a. **Greenberg, A. H., and M. Greene.** 1976. Non-adaptive rejection of small tumour inocula as a model of immune surveillance. Nature (London) 264:356–359.

469b. **Gregonis, D. E., and H. C. Rilling.** 1974. The stereochemistry of *trans*-phytoene synthesis. Some observations on lycopersene as a carotene precursor and a mechanism for the synthesis of *cis*- and *trans*-phytoene. Biochemistry 13:1538–1542.

470. **Grellert, E., and C. E. Ballou.** 1972. Biosynthesis of a mycobacterial lipopolysaccharide. Evidence for an acylpolysaccharide methyltransferase. J. Biol. Chem. 247:3236–3241.
471. **Griffith, A. S.** 1925. The serological classification of mammalian and avian tubercle bacilli. Tubercle 6:417–436.
472. **Griffith, A. S.** 1937. Bovine tuberculosis in man. Tubercle (London) 18:529–543.
473. **Grillo-Lopez, A. J., E. Rivera, M. Castillo-Staab, and N. Maldonado.** 1971. Disseminated *M. kansasii* infection in a patient with chronic granulocytic leukemia. Cancer 28:476–481.
474. **Grosset, J., G. Decroix, and C. Sors.** 1971. Les tuberculoses a *Mycobacterium africanum* chez les nois africains de la region Parisienne. Rev. Tuberc. Pneumonol. 35:430–436.
475. **Gruft, H., and H. G. Henning.** 1972. Pulmonary mycobacteriosis due to rapidly growing acid-fast bacillus, *Mycobacterium chelonei*. Am. Rev. Respir. Dis. 105:618–620.

476. Gruft, H., and A. Loder. 1971. Enhancing effects of carbon dioxide on the primary isolation of acid-fast bacilli in a modified Lowenstein-Jensen medium. Appl. Microbiol. 22:944–945.
477. Grunberg, E. (ed.) 1963. Current status of tuberculosis. Ann. N. Y. Acad. Sci. 106:1–156.
478. Gunnels, J. J., and J. H. Bates. 1972. Characterization and mycobacteriophage typing of *Mycobacterium xenopi*. Am. Rev. Respir. Dis. 105:388–396.
479. Gunthorpe, W. J., and J. L. Stanford. 1972. A study of *Mycobacterium gordonae* and *Mycobacterium marianum* (*scrofulaceum*). Br. J. Exp. Pathol. 53:665–671.
480. Habib, N. I., and F. C. Warring, Jr. 1966. A fatal case of infection due to *Mycobacterium bovis*. Am. Rev. Respir. Dis. 93:804–810.
481. Hagan, W. A., and W. A. Zeissig. 1929. Johnin versus avian tuberculin as a diagnostic agent for paratuberculosis (Johne's disease) of cattle. J. Am. Vet. Med. Assoc. 74:985–1003.
482. Hagemann, P. K. H. 1937. Fluoreszenzmikroskopische Untersuchungen über Virus- und andere Mikroben. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Beihft. 140:184–187.
483. Hagihara, Y., and K. Shikada. 1969. The base composition of deoxyribonucleic acid isolated from mycobacteria (in Japanese). Kekkaku 44:321–324.
484. Hagmar, B., J. Kutti, P. Lundin, M. Norlin, A. Weinfeld, and P. Wahlen. 1969. Disseminated infection caused by *Mycobacterium kansasii*. Report of a case and brief review of the literature. Acta Med. Scand. 186:93–99.
485. Hahn, H. 1974. Effects of dextran sulfate 500 on cell-mediated resistance to infection with *Listeria monocytogenes* in mice. Infect. Immun. 10:1105–1109.
486. Hahn, H., and M. Bierther. 1974. Morphological changes induced by dextran sulfate 500 in mononuclear phagocytes of *Listeria*-infected mice. Infect. Immun. 10:1110–1119.
487. Hand, W. L., and J. P. Sanford. 1970. *Mycobacterium fortuitum*—a human pathogen. Ann. Intern. Med. 73:971–977.
488. Hanks, J. H., and B. Evans. 1940. The action of serum, cells and blood or acid-fast bacteria *in vitro*. I. Absence of *in vitro* bactericidal power against human tubercle bacilli or timothy bacilli in the serum-leucocyte mixtures or the blood of normal and immunized rabbits. Am. Rev. Tuberc. 41:605–619.
489. Hansen, G. A. 1955. Spedalskhadens arsagen (causes of leprosy), reprinted in translation from Norsk Magazin for Laegevidenskaben 4 (1874), p. 76–79. Int. J. Lepr. 23:307–309.
490. Harada, K. 1973. Effect of prior oxidation on the acid-fastness of mycobacteria. Stain Technol. 48:269–273.
491. Harrington, B. J. 1966. A numerical taxonomical study of some corynebacteria and related organisms. J. Gen. Microbiol. 45:31–40.
492. Harrison, S. C., D. L. D. Caspar, R. D. Camerini-Otero, and R. M. Franklin. 1971. Lipid and protein arrangement in bacteriophage PM2. Nature (London) New Biol. 229:197–201.
493. Hart, P. D. 1967. Efficacy and applicability of mass B. C. G. vaccination in tuberculosis control. Br. Med. J. 1:587–592.
494. Hart, P. D., and J. A. Armstrong. 1974. Strain virulence and the lysosomal response in macrophages infected with *Mycobacterium tuberculosis*. Infect. Immun. 10:742–746.
495. Hart, P. D., J. A. Armstrong, C. A. Brown, and P. Draper. 1972. Ultrastructural study of the behavior of macrophages toward parasitic mycobacteria. Infect. Immun. 5:803–807.
496. Hart, P. D., A. H. Gordon, and P. J. Jacques. 1969. Suggested role of lysosomal lipid in the contrasting effects of "Triton WR-1339" and dextran on tuberculous infection. Nature (London) 222:672–673.
497. Hart, P. D., T. M. Pollock, and I. Sutherland. 1957. Assessment of the first results of the Medical Research Council's trial of tuberculosis vaccines in adolescents in Great Britain. Adv. Tuberc. Res. 8:171–189.
498. Hartwigk, H., and P. Hummel. 1967. Prüfung von Mykobakterien auf Variabilität nach Selektion mit der Einzellkultur. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. I Abt. Orig. 202:487–496.
499. Hatt, H. D., E. F. Lessel, W. A. Clark, E. E. Davis, S. C. Jong, R. G. Zieg, and M. T. Alexander. 1974. American Type Culture Collection catalogue of strains, 11th ed. Rockville, Md.
500. Hauduroy, P. 1955. Derniers aspects du monde des mycobacteries. Masson et Cie, Paris.
501. Hayes, W. 1968. The genetics of bacteria and their viruses. Studies in basic genetics and molecular biology, 2nd ed. Blackwell Scientific Publications. Oxford.
502. Heise, E. R., S. Han, and R. S. Weiser. 1968. *In vitro* studies on the mechanism of macrophage migration inhibition in tuberculin sensitivity. J. Immunol. 101:1004–1015.
503. Hepper, N. G. G., A. G. Karlson, F. J. Leary, and E. H. Soule. 1971. Genitourinary infection due to *Mycobacterium kansasii*. Proc. Mayo Clin. 46:387–390.
503a. Herberman, R. B., M. E. Nunn, H. T. Holden and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors II. Characterization of effector cells. Int. J. Cancer 16:230–239.
503b. Herberman, R. B., M. E. Nunn, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of

reactivity and specificity. Int. J. Cancer 16:216–229.

504. **Herndon, J. H., D. R. Dantzker, and A. M. Lanoue.** 1972. *Mycobacterium fortuitum* infections involving the extremities. Report of three cases. J. Bone Jt. Surg. 54A:1279–1282.
505. **Hertzberg, S., and S. Liaaen-Jensen.** 1966. Bacterial carotenoids. XIX. The carotenoids of *Mycobacterium phlei* strain Vera. 1. The structure of the minor carotenoids. Acta Chem. Scand. 20:1187–1194.
506. **Hertzberg, S., and S. Liaaen-Jensen.** 1967. Bacterial carotenoids. XX. The carotenoids of *Mycobacterium phlei* strain Vera. 2. The structures of the phlei-xanthophylls—two novel tertiary glucosides. Acta Chem. Scand. 21:15–41.
507. **Herzenberg, L. A., and L. A. Herzenberg.** 1974. Short term and chronic allotype suppression in mice, p. 41–75. *In* M. D. Cooper and N. L. Warner (ed.), Contemporary topics in immunobiology, vol. 3. Plenum Press, New York.
508. **Herzenberg, L. A., C. M. Metzler, and L. A. Herzenberg.** 1974. Mechanism of allotype suppression in mice, p. 519–529. *In* D. H. Katz and B. Benacerraf (ed.), Immunological tolerance; mechanisms and potential therapeutic applications. Academic Press Inc., New York.
509. **Hesselberg, I.** 1972. Drug resistance in the Swedish/Norwegian BCG strain. Bull W.H.O. 46:503–507.
510. **Hetey, L., E. Vandra, and I. Foldes.** 1968. In vitro synthesis of antibody to phages of *Mycobacterium phlei*. Acta Microbiol. Acad. Sci. Hung. 15:125–127.
511. **Higashi, Y., J. L. Strominger, and C. C. Sweeley.** 1967. Structure of a lipid intermediate in cell wall peptidoglycan synthesis: a derivative of a C_{55} isoprenoid alcohol. Proc. Natl. Acad. Sci. U.S.A. 57:1878–1884.
512. **Hill, D. L., and C. E. Ballou.** 1966. Biosynthesis of mannophospholipids by *Myocbacterium phlei*. J. Biol. Chem. 241:895–902.
513. **Hill, E. B., L. G. Wayne, and W. M. Gross.** 1972. Purification of mycobacterial deoxyribonucleic acid. J. Bacteriol. 112:1033–1039.
514. **Hill, G. J.** 1970. Leprosy in five young men. Colorado Associated University Press, Boulder.
515. **Hobby, G. L., W. B. Redmond, E. H. Runyon, W. B. Schaefer, L. G. Wayne, and R. H. Wickelhausen.** 1967. A study on pulmonary diseases associated with mycobacteria other than *Mycobacterium tuberculosis:* identification and characterization of the mycobacteria. XVIII. Rep. Vet. Admin.-Armed Forces Coop. Study 95:954–971.
516. **Holland, J. J., and M. J. Pickett.** 1958. A cellular basis of immunity in experimental brucella infection. J. Exp. Med. 108:343–359.
517. **Holmes, R. K., and L. Barksdale.** 1969. Genetic analysis of tox^+ and tox^- bacteriophages of *Corynebacterium diphtheriae*. J. Virol. 3:586–598.
518. **Holmes, R. K., and L. Barksdale.** 1970. Comparative studies with tox^+ and tox^- corynebacteriophages. J. Virol. 5:783–794.
519. **Holt, J. G., secretary, Judicial Commission.** 1974. Opinion 51. Conservation of the epithet *fortuitum* in the combination *Mycobacterium fortuitum* da Costa Cruz. Int. J. Syst. Bacteriol. 24:552.
520. **Hopwood, D. A., K. F. Chater, J. E. Dowding, and A. Vivian.** 1973. Advances in *Streptomyces coelicolor* genetics. Bacteriol. Rev. 37:371–405.
521. **Horwitz, O., and J. Meyer.** 1957. The safety record of BCG vaccination and untoward reactions observed after vaccination. Adv. Tuberc. Res. 8:245–271.

521a. **Houbouyan, L., J. Soria, C. Soria, and A. Goguel.** 1974. Analysis of an autoimmune antibody (IgM class) in a case of chronic lymphocytic leukaemia. Haemostasis 3:171–182.

522. **Houchens, D. P., A. I. Goldberg, M. R. Gaston, M. Kende, and A. Goldin.** 1973. Studies of the effects of *Bacillus Calmette-Guérin* on Moloney sarcoma virus-induced tumors in normal and immunosuppressed mice. Cancer Res. 33:685–690.
523. **Howes, C. D., and P. P. Batra.** 1970. Accumulation of lycopene and inhibition of cyclic carotenoids in Mycobacterium in the presence of nicotine. Biochim. Biophys. Acta 222:174–179.
524. **Howes, C. D., and P. P. Batra.** 1970. Mechanism of photoinduced carotenoid synthesis. Further studies on the action spectrum and other aspects of carotenogenesis. Arch. Biochem. Biophys. 137:175–180.
525. **Howes, C. D., P. P. Batra, and C. F. Blakeley.** 1969. Absolute requirement for oxygen during illumination for photoinduced carotenoid synthesis. Biochim. Biophys. Acta 189:298–299.
526. **Huempfner, H. R., W. R. Kingsolver, and K. W. Deuschle.** 1966. Tuberculous meningitis caused by both *Mycobacterium tuberculosis* and atypical mycobacteria. Am. Rev. Respir. Dis. 94:612–614.
527. **Huet, M., N. Rist, G. Boube, and D. Potier.** 1971. Etude bacteriologique de la tuberculose au Cameroun. Rev. Tuberc. (Paris) 35:413–426.
528. **Hughes, D. E., E. S. Moss, M. Hood, and M. Henson.** 1954. Virulence of *Mycobacterium tuberculosis*. Evaluation of a test, using neutral red indicator. Am. J. Clin. Pathol. 24:621–625.
529. **Ilton, M., A. W. Jevans, E. D. McCarthy, D. Vance, H. B. White III, and K. Bloch.** 1971. Fatty acid synthetase activity in *Mycobacterium phlei:* regulation by polysaccharides. Proc. Natl. Acad. Sci. U.S.A. 68:87–91.
530. **Imaeda, T.** 1965. Electron microscopy. Ap-

proach to leprosy research. Int. J. Lepr. 33:669–688.
531. Imaeda, T., and M. Rieber. 1968. Mitomycin C-induced phage-like particles in a mutant of *Mycobacterium tuberculosis* BCG. J. Bacteriol. 96:557–559.
532. Imaeda, T., and M. Rieber. 1970. Defective cell-wall formation associated with lysogeny in mycobacteria, p. 189–203. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium*, *Nocardia* and *Actinomyces*. Charles C Thomas, Springfield, Ill.
533. Imaeda, T., and F. San Blas. 1969. Adsorption of mycobacteriophage on cell-wall components. J. Gen. Virol. 5:493–498.
534. Ingraham, M. A., and H. Steenbock. 1935. The relation of microorganisms to carotenoids and vitamin A. II. The production of carotenoids by *Mycobacterium phlei*. Biochem. J. 29:2553–2562.
535. Inman, P. M., A. Beck, A. E. Brown, and J. L. Stanford. 1969. Outbreak of injection abscesses due to *Mycobacterium abscessus*. Arch. Dermatol. 100:141–147.
536. Ioneda, T., E. Lederer, and J. Rozanis. 1970. Sur la structure des diesters de tréhalose ("cord factors") produits par *Nocardia asteroides* et *Nocardia rhodocrous*. Chem. Phys. Lipids 4:375–392.
537. Ioneda, T., M. Lenz, and J. Pudles. 1963. Chemical constitution of a glycolipid from *C. diphtheriae* PW-8. Biochem. Biophys. Res. Commun. 13:110–114.
538. Ishibashi, T., Y. Fujiwara, A. Tanaka, and K. Sugiyama. 1969. Antigenicity of wax D. IV. Its relationship to other hemagglutination reactions. Int. Arch. Allergy Appl. Immunol. 36:506–514.
539. Ishibashi, T., D. Hagimoto, S. Shinozaki, A. Tanaka, and K. Sugiyama. 1971. Immunological adjuvants. V. A critical evaluation of minimal requirements for mycobacterial adjuvants to induce delayed hypersensitivity. Jpn. J. Exp. Med. 41:95–102.
540. Iturribarria, F. M., and G. Middlebrook. 1953. Un caso de infection humana por *Mycobacterium ulcerans* en el hemisfero occidental nota previa. Int. J. Lepr. 21:469–476.
541. Jacob, F., and E. L. Wollman. 1955. Étude génétique d'un bactériophage tempéré d'*Escherichia coli*. III. Effect du rayonnement ultraviolet sur la recombinaison génêtique. Ann. Inst. Pasteur (Paris) 88:724–749.
542. Jacob, F., and E. L. Wollman. 1961. Sexuality and the genetics of bacteria. Academic Press Inc., New York.
543. Jahn, R. P. 1959. Silicosis and avian tuberculosis. Am. Rev. Respir. Dis. 80:78–84.
544. Janicki, B. W., S. A. Aron, G. P. Schechter, and W. McFarland. 1972. Tuberculin antigens active in human lymphocyte blastogenesis. Proc. Soc. Exp. Biol. Med. 141:809–813.
545. Janicki, B. W., S. D. Chaparas, T. M. Daniel, G. P. Kubica, G. L. Wright Jr., and G. S. Yee. 1971. A reference system for antigens of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 104:602–604.
546. Jauréguiberry, G., J. H. Law, J. A. McCloskey, and E. Lederer. 1965. Studies on the mechanism of biological carbon alkylation reactions. Biochemistry 4:347–353.
547. Jauréguiberry, G., M. Lenfant, B. C. Das, and E. Lederer. 1966. Sur le mécanisme des C-méthylations biologiques par la methionine. Tetrahedron 22(Suppl. 8):27–32.
548. Jauréguiberry, G., M. Lenfant, R. Toubiana, R. Azerad, and E. Lederer. 1966. Biosynthesis of tuberculostearic acid in a cell-free extract. Identification of 10-methylenestearic acid as an intermediate. Chem. Commun., p. 855–857.
549. Jenkins, D. E., and E. Wolinsky. 1974. Mycobacterial diseases of the lung and bronchial tree: treatment of active pulmonary tuberculosis, p. 323–365. *In* G. L. Baum (ed.), Textbook of pulmonary diseases, 2nd ed. Little, Brown and Co., Boston.
550. Jenkins, P. A., J. Marks, and W. B. Schaefer. 1971. Lipid chromatography and seroagglutination in the classification of rapidly growing mycobacteria. Am. Rev. Respir. Dis. 103:179–187.
551. Johanson, W. G., Jr., and D. P. Nicholson. 1969. Pulmonary disease due to *Mycobacterium kansasii*. An analysis of some factors affecting prognosis. Am. Rev. Respir. Dis. 99:73–85.
552. Johne, H. A., and L. Frothingham. 1895. Ein eigenthumlichen Fall von Tuberculose beim Rind. Deutsch. Z. Tiermed. Vergl. Pathol. 21:438–454.
553. Johnson, J. H., B. C. Reed, and H. C. Rilling. 1974. Early photoinduced enzymes of photoinduced carotenogenesis in a *Myocbacterium* species. J. Biol. Chem. 249:402–406.
554. Jollès, P., F. Bigler, T. Gendre, and E. Lederer. 1961. Sur la structure chimique du "mycoside C", peptidoglycolipide de *Mycobacterium avium*. Bull. Soc. Chim. Biol. 43:177–192.
555. Jollès, P., D. Samour, and E. Lederer. 1962. Analytical studies on wax D, a macromolecular peptido-glycolipid fraction from human strains of *Mycobacterium tuberculosis*. Arch. Biochem. Biophys. 99:283–289.
556. Jollès, P., D. Samour, and E. Lederer. 1963. Isolement de fractions peptido-glycolipidiques à partir des cires D de mycobactéries bovines, atypiques, aviaires et saprophytes. Biochim. Biophys. Acta 78:342–350.
557. Jollès, P., D. Samour-Migliore, H. De Wijs, and E. Lederer. 1964. Correlation of adjuvant activity and chemical structure of mycobacterial wax D fractions: the importance of amino sugars. Biochim. Biophys. Acta 83:361–363.
558. Jolly, H. W., and J. H. Seabury. 1972. Infections with *Mycobacterium marinum*. Arch. Dermatol. 106:32–36.

559. **Jones, T., and G. P. Youmans.** 1974. Nonspecific inhibition of growth of intracellular *Listeria monocytogenes* by lymphocyte culture products. Infect. Immun. 9:472–474.
560. **Jones, T. D., and J. R. Mote.** 1934. The phases of foreign protein sensitization in human beings. N. Engl. J. Med. 210:120–123.
561. **Jones, W. D., Jr., V. D. Abbot, A. L. Vestal, and G. P. Kubica.** 1966. A hitherto undescribed group of nonchromogenic mycobacteria. Am. Rev. Respir. Dis. 94:790–795.
562. **Jones, W. D., Jr., and H. L. David.** 1970. Biosynthesis of a lipase by *Mycobacterium smegmatis* ATCC 607 infected by mycobacteriophage D29. Am. Rev. Respir. Dis. 102:818–820.
563. **Jones, W. D., and H. L. David.** 1972. Preliminary observations on the occurrence of a streptomycin R-factor in *Mycobacterium smegmatis* ATCC 607. Tubercle 53:35–42.
564. **Jones, W. D., H. L. David, and R. E. Beam.** 1970. The occurrence of lipids in mycobacteriophage D29 propagated in *Mycobacterium smegmatis* ATCC 607. Am. Rev. Respir. Dis. 102:814–817.
565. **Jopling, W. H.** 1971. Handbook of leprosy. William Heinemann Medical Books, Ltd., London.
566. **Juhasz, S. E., and R. Bönicke.** 1965. Possible classification of rapidly growing mycobacteria on the basis of their phage susceptibility. Can. J. Microbiol. 11:235–241.
567. **Juhasz, S. E., and G. Plummer (ed.).** 1970. Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Springfield, Ill.
568. **Juhlin, I.** 1967. Contribution to the classification of mycobacteria and nocardias. Acta Pathol. Microbiol. Scand. 70(Suppl. 189):1–120.
569. **Juhlin, I., and S. Winblad.** 1973. Sero-typing of mycobacteria by a new technique using antibody globulin absorbed to staphylococcal protein A. Acta Pathol Microbiol. Scand. B. 81:179–180.
570. **Kahn, M. C.** 1929. A developmental cycle of the tubercle bacillus as revealed by single-cell studies. Am. Rev. Tuberc. 20:150–200.
571. **Kanai, K.** 1967. Affinity of lysosomal acid phosphatase to the surface of the tubercle bacillus. Jpn. J. Med. Sci. Biol. 20:401–411.
572. **Kanai, K.** 1967. Detection of host-originated acid phosphatase on the surface of "*in vivo* grown tubercle bacilli." Jpn. J. Med. Sci. Biol. 20:73–90.
573. **Kanai, K.** 1967. Resistance to sodium hydroxide treatment of "*in vivo* grown tubercle bacilli." Jpn. J. Med. Sci. Biol. 20:91–96.
574. **Kanai, K., and E. Kondo.** 1970. A suggested role of the lysosomal membrane as a part of the defence mechanism against tuberculous infection. Jpn. J. Med. Sci. Biol. 23:295–302.
575. **Kanai, K., E. Wiegeshaus, and D. W. Smith.** 1970. Demonstration of mycolic acid and phthiocerol dimycocerosate in "*in vivo* grown tubercle bacilli." Jpn. J. Med. Sci. Biol. 23:327–333.
576. **Kanemasa, Y., and D. S. Goldman.** 1965. Direct incorporation of octanoate into long-chain fatty acids by soluble enzymes of *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 98:476–485.
577. **Kanetsuna, F.** 1968. Chemical analyses of mycobacterial cell walls. Biochim. Biophys. Acta 158:130–143.
578. **Kanetsuna, F., and G. San Blas.** 1970. Chemical analysis of a mycolic acid-arabinogalactan-mucopeptide complex of mycobacterial cell wall. Biochim. Biophys. Acta 208:434–443.
579. **Kantzler, G. B., S. F. Lauteria, C. L. Cusumano, J. D. Lee, R. Ganguly, and R. H. Waldman.** 1974. Immunosuppression during influenza virus infection. Infect. Immun. 10:996–1002.
580. **Kaplan, H., and M. Clayton.** 1969. Carpal tunnel syndrome secondary to *Mycobacterium kansasii* infection. J. Am. Med. Assoc. 208:1186–1188.
581. **Käppler, W.** 1961. Uber die Lysotypie von saprophytaren Mycobakterien. Naturwissenschaften 48:630–631.
582. **Karassova, V., J. G. Weiszfeiler, and E. Krasznay.** 1965. Occurrence of atypical mycobacteria in *Macacus rhesus*. Acta Microbiol. Acad. Sci. Hung. 12:275–282.
583. **Karczag, E., and J. G. Weiszfeiler.** 1973. Antigenic structure of *M. simiae* and *M. asiaticum*, p. 175–186. *In* J. G. Weiszfeiler (ed.), Atypical mycobacteria. Akademiai Kiado, Budapest.
584. **Karlson, A. G., and D. T. Carr.** 1970. Tuberculosis caused by *Mycobacterium bovis*. Report of six cases: 1954–1968. Ann. Intern. Med. 73:979–983.
585. **Karlson, A. G., and E. F. Lessel.** 1970. *Mycobacterium bovis* nom. nov. Int. J. Syst. Bacteriol. 20:273–282.
586. **Kasten, F. H.** 1960. The chemistry of Schiff's reagent. Int. Rev. Cytol. 10:1–100.
587. **Katagiri, M., S. Takemori, T. Oda, and T. Matsumoto.** 1974. A lactate oxygenase from *Mycobacterium pheli*. III. Evidence for the subunit structure. Electron microscopic studies—"morphology." Arch. Biochem. Biophys. 160:295–303.
588. **Kataoka, T., and S. Nojima.** 1967. The phospholipid composition of some actinomycetes. Biochim. Biophys. Acta 144:681–683.
589. **Kataria, Y. P.** 1969. Observations on human infection with *Mycobacterium bovis*. Tubercle (London) 50:14–21.
590. **Kato, K., J. L. Strominger, and S. Kotani.** 1968. Structure of the cell wall of *Corynebacterium diphtheriae*. I. Mechanism of hydrolysis by the L-3 enzyme and the structure of the peptide. Biochemistry 7:2762–2773.
591. **Kato, M.,** 1968. Studies of a biochemical lesion in experimental tuberculosis in mice. VIII.

Effect of derivatives and chemical analogues of cord factor on structure and function of mouse liver mitochondria. Am. Rev. Respir. Dis. 98:668–676.

592. **Kato, M.** 1970. Site II-specific inhibition of mitochondrial oxidative phosphorylation by trehalose-6,6'-dimycolate (cord factor) of *Mycobacterium tuberculosis*. Arch. Biochem. Biophys. 140:379–390.
593. **Kato, M.** 1971. The role of cord factor in pathogenesis of tuberculosis. Jpn. J. Med. Sci. Biol. 24:47–51.
594. **Kato, M.** 1972. Antibody formation to trehalose-6,6'-dimycolate (cord factor) of *Mycobacterium tuberculosis*. Infect. Immun. 5:203–212.
595. **Kato, M.** 1973. Effect of anti-cord factor antibody on experimental tuberculosis in mice. Infect. Immun. 7:14–21.
596. **Kato, M.** 1973. Immunochemical properties of anti-cord factor antibody. Infect. Immun. 7:9–13.
597. **Kato, M.** 1974. Further study of neutralization of biochemical activity of cord factor by anti-cord factor antibody. Infect. Immun. 10:277–279.
598. **Kato, M., and J. Asselineau.** 1971. Chemical structure and biochemical activity of cord factor analogs. 6,6'-Dimycoloyl sucrose and methyl 6-mycoloyl-α-D-glucoside. Eur. J. Biochem. 22:364–370.
599. **Kato, M., and M. B. Goren.** 1974. Synergistic action of cord factor and mycobacterial sulfatides on mitochondria. Infect. Immun. 10:733–741.
600. **Kato, M., and J. Maeda.** 1974. Isolation and biochemical activities of trehalose-6-monomycolate of *Mycobacterium tuberculosis*. Infect. Immun. 9:8–14.
601. **Kato, M., and A. Tanaka.** 1967. Studies of a biochemical lesion in experimental tuberculosis in mice. V. Further study on a toxic lipid fraction in firmly bound lipids. Am. Rev. Respir. Dis. 96:460–468.
602. **Katz, D. H., and B. Benacerraf.** 1972. The regulatory influence of activated T cells on B cell responses to antigen. Adv. Immunol. 15:1–94.
603. **Katz, D. H., and B. Benacerraf (ed.).** 1974. Immunological tolerance. Mechanisms and potential therapeutic applications. Academic Press Inc., New York.
604. **Kazda, J.** 1967. Mycobacterien im Trinkwasser als Ursache der Parallergie gegenüber Tuberkulinen bei Tieren. III. Mitteilung: Taxonomische Studie einiger rasch wachsender Mykobakterien und Beschreibung einer neuen Art: *Mycobacterium brunense* n. sp. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. I. Abt. Orig. 203:199–211.
605. **Kedrowski, W. J.** 1901. Ueber die Cultur der Lepraerreger. Z. Hyg. Infektionskr. 37:52–69.
606. **Kellenberger, E.** 1961. Vegetative bacteriophage and the maturation of the virus particles, p. 1–61. *In* K. M. Smith and M. A. Lauffer (ed.), Advances in virus research, vol. 8. Academic Press Inc., New York.
607. **Keller, J. M., and C. E. Ballou.** 1968. The 6-*O*-methylglucose-containing lipopolysaccharide of *Mycobacterium phlei*. Identification of the lipid components. J. Biol. Chem. 243:2905–2910.
608. **Keller, R. H., and E. H. Runyon.** 1964. Mycobacterial diseases. Am. J. Roentgenol. Radium Ther. Nucl. Med. 92:528–539.
609. **Keller-Schierlein, W., and V. Prelog.** 1961. Über das Ferrioxamin E; ein Beitrag zur Konstitution des Nocardamins. Helv. Chim. Acta 44:1981–1985.
610. **Khuller, G. K., and P. J. Brennan.** 1972. Further studies on the lipids of corynebacteria. The mannolipids of *Corynebacterium aquaticum*. Biochem. J. 127:369–373.
611. **Khuller, G. K., and P. J. Brennan.** 1972. The mannophosphoinositides of the unclassified *Mycobacterium* P_6. Am. Rev. Respir. Dis. 106:892–896.
612. **Khuller, G. K., and P. J. Brennan.** 1972. The polar lipids of some species of *Nocardia*. J. Gen. Microbiol. 73:409–412.
613. **Khuller, G. K., and D. Subrahmanyam.** 1971. Antigenicity of phosphatidyl inositomannosides of *Mycobacterium tuberculosis*. Immunochemistry 8:251–256.
613a. **Kiessling, R., E. Klein, H. Pross, and H. Wigzell.** 1975. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur. J. Immunol. 5:117–121.
613b. **Kiessling, R., G. Petranyi, K. Karre, M. Jondal, D. Tracey, and H. Wigzell.** 1976. Killer cells: a functional comparison between natural, immune T-cell and antibody-dependent in vitro systems. J. Exp. Med. 143:772–780.
614. **Kim, K.-S., M. R. J. Salton, and L. Barksdale.** 1976. Ultrastructure of superficial mycosidic integuments of *Mycobacterium* sp. J. Bacteriol. 125:739–743.
615. **Kincade, G. F.** 1959. Avian tuberculosis in the human. Can. Med. Assoc. J. 80:32–34.
616. **Klebanoff, S. J.** 1970. Myeloperoxidase: contribution to the microbicidal activity of intact leukocytes. Science 169:1095–1097.
617. **Kleinig, H.** 1974. Inhibition of carotenoid synthesis in *Myxococcus fulvus* (*Myxobactales*). Arch. Mikrobiol. 97:217–226.
618. **Klun, C. L., R. G. Neiburger, and G. P. Youmans.** 1973. Relationship between mouse mycobacterial growth-inhibitory factor and mouse migration-inhibitory factor in supernatant fluids from mouse lymphocyte cultures. J. Reticuloendothel. Soc. 13:310–318.
619. **Klun, C. L., and G. P. Youmans.** 1973. The effect of lymphocyte supernatant fluids on the intracellular growth of virulent tubercle bacilli. J. Reticuloendothel. Soc. 13:263–274.

620. **Klun, C. L., and G. P. Youmans.** 1973. The induction of *Listeria monocytogenes* and plant mitogens of lymphocyte supernatant fluids which inhibit the growth of *Myobacterium tuberculosis* within macrophages *in vitro*. J. Reticuloendothel. Soc. 13:275-285.

621. **Knoche, H., T. W. Esders, K. Koths, and K. Bloch.** 1973. Palmityl coenzyme A inhibition of fatty acid synthesis. Relief by bovine serum albumin and mycobacterial polysaccharides. J. Biol. Chem. 248:2317-2322.

622. **Knoche, H. W., and K. E. Koths.** 1973. Characterization of a fatty acid synthetase from *Corynebacterium diphtheriae*. J. Biol. Chem. 248:3517-3519.

623. **Koch, R.** 1882. Die Aetiologie der Tuberkulose. Berl. Klin. Wochenschr. 19:221-230.

624. **Koch, R.** 1897. Ueber neue Tuberkulinpräparate. Dtsch. Med. Wochenschr. 23:209-213.

625. **Kochan, I.** 1973. The role of iron in bacterial infections, with special consideration of host-tubercle bacillus interaction. Curr. Top. Microbiol. Immunol. 60:1-30.

626. **Kochan, I., and C. A. Golden.** 1974. Immunological nature of antimycobacterial phenomenon in macrophages. Infect. Immun. 9:249-254.

627. **Koch-Weser, D., R. H. Ebert, W. R. Barclay, and V. S. Lee.** 1953. Studies on the metabolic significance of acid-fastness of tubercle bacilli. J. Lab. Clin. Med. 42:828-829.

628. **Koenig, M. G., R. D. Collins, and R. M. Heyssel.** 1966. Disseminated mycobacteriosis caused by Battey type mycobacteria. Ann. Intern. Med. 64:145-154.

629. **Kölbel, H.** 1970. Phage multiplication in mycobacterium as a function of time and multiplicity of infection: an electron microscopic study, p. 29-38. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium*, *Nocardia* and *Actinomyces*. Charles C Thomas, Springfield, Ill.

630. **Kölbel, H. K., and H. Mohelská.** 1971. Versuche zur morphologischen Charakterisierung einiger Mykobakteriophagen (AG1, GS4E, D29, BK1). Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A 217:507-528.

631. **Kolmanova, A., J. Hochmannova, and I. Malek.** 1970. Carotenoids synthesized by UV-induced mutants of a non-acid-fast strain of *Mycobacterium phlei*. Folia Microbiol. (Prague) 15:426-430.

632. **Komura, I., K. Yamada, S.-I. Otsuka, and K. Komagata.** 1975. Taxonomic significance of phospholipids in coryneform and nocardioform bacteria. J. Gen. Appl. Microbiol. 21:251-261.

633. **Komuro, K., and E. A. Boyse.** 1973. In-vitro demonstration of thymic hormone in the mouse by conversion of precursor cells into lymphocytes. Lancet 1:740-743.

634. **Kondo, E., and K. Kanai.** 1972. Separation of tuberculin-active proteins from the infected mouse lung tissue. Jpn. J. Med. Sci. Biol. 25:249-257.

635. **Kondo, E., and K. Kanai.** 1974. A comparative observation on cholesterol ester contents of uninduced and induced mouse peritoneal cells. Jpn. J. Med. Sci. Biol. 27:67-79.

636. **Kondo, E., and K. Kanai.** 1974. Further studies on the increase in cholesterol ester content of the lungs of tuberculous mice. Jpn. J. Med. Sci. Biol. 27:59-65.

637. **Kondo, E., and K. Kanai.** 1974. Some immunological and toxic properties of mycobacteria grown in vivo. Jpn. J. Med. Sci. Biol. 27:53-57.

638. **Kondo, E., K. Kanai, K. Nishimura, and T. Tsumita.** 1970. Analysis of host-originated lipids associating with "in vivo grown tubercle bacilli." Jpn. J. Med. Sci. Biol. 23:315-326.

639. **Koníčková-Radochová, M., and I. Málek.** 1969. The mutagenic effect of nitrosoguanidine on *Mycobacterium phlei* PA. Folia Microbiol. (Prague) 14:201-207.

640. **Koníčková-Radochová, M., and I. Málek.** 1969. The induction of auxotrophic mutants of *Mycobacterium phlei* PA by ultraviolet irradiation. Folia Microbiol. (Prague) 14:251-255.

641. **Koníčková-Radochová, M., and I. Málek.** 1969. The use of ethyl methanesulfonate for the induction of mutants in *Mycobacterium phlei* strain PA. Folia Microbiol. (Prague) 14:470-474.

642. **Konno, K., K. Oizumi, F. Ariji, J. Yamaguchi, and S. Oka.** 1973. Mode of action of rifampin on mycobacteria. 1. Electron microscopic study of the effect of rifampin on *Mycobacterium tuberculosis*. Am. Rev. Resp. Dis. 107:1002-1005.

643. **Koopman, W. J., M. H. Gillis, and J. R. David.** 1973. Prevention of MIF activity by agents known to increase cellular cyclic AMP. J. Immunol. 110:1609-1614.

644. **Korn, O.** 1899. Zur Kenntnis der Säurefesten Bakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 25:532-541.

645. **Kornhauser, A., N. I. Krinsky, P.-K. C. Huang, and D. C. Clagett.** 1973. A comparative study of photodynamic oxidation and radio frequency-discharge-generated $^1O_2$ oxidation of guanosine. Photochem. Photobiol. 18:63-69.

646. **Korsak, T.** 1975. Occurrence of L-forms in a case of generalized mycobacteriosis due to *Mycobacterium scrofulaceum*. Acta Tuberc. Pneumol. Belg. 66:445-469.

647. **Korsak, T., and H. Boisvert.** 1972. Mycobactéries a pigment rose. Ann. Inst. Pasteur Paris 122:31-41.

648. **Kostiala, A. A. I.** 1971. Delayed hypersensitivity in the guinea pig immunized with killed tubercle bacilli in adjuvant. 1. Development of peritoneal cell migration inhibition, skin reactions and antibodies to tuber-

culin purified protein derivative. Acta Pathol. Microbiol. Scand. Sect. B 79:275-280.
649. **Kostiala, A. A. I.** 1971. Delayed hypersensitivity in the guinea pig immunized with killed tubercle bacilli in adjuvant. 2. The effect of tuberculin purified protein derivative concentration on the inhibition of peritoneal cell migration. Acta Pathol. Microbiol. Scand. Sect. B 79:281-284.
650. **Kostiala, A. A. I., and T. U. Kosunen.** 1972. Delayed hypersensitivity in the guinea pig immunized with killed tubercle bacilli in adjuvant. III. Peritoneal cell migration inhibition and macrophage cytophilic antibodies to tuberculin purified protein derivative. Acta Pathol. Microbiol. Scand. Sect. B 80:1-9.
651. **Kotani, S., S. Hashimoto, T. Matsubara, K. Kato, K. Harada, J. Kogami, T. Kitaura, and A. Tanaka.** 1963. Lysis of isolated BCG cell walls with enzymes. II. Demonstration of "bound wax D" as a component of BCG cell walls. Biken J. 6:181-196.
652. **Kotani, S., T. Kato, T. Matsuda, K. Kato, and A. Misaki.** 1971. Chemical structure of the antigenic determinants of cell wall polysaccharide of *Mycobacterium tuberculosis* strain H37Rv. Biken J. 14:379-387.
653. **Kotani, S., T. Kitaura, T. Hirano, and S. Tanaka.** 1959. Isolation and chemical composition of the cell walls of BCG. Biken J. 2:129-141.
654. **Kotani, S., T. Narita, D. E. S. Stewart-Tull, T. Shimono, Y. Watanabe, K. Kato, and S. Iwata.** 1975. Immunoadjuvant activities of cell walls and their water-soluble fractions prepared from various gram-positive bacteria. Biken J. 18:77-92.
655. **Kotani, S., Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka.** 1975. Immunoadjuvant activities of synthetic *N*-acetylmuramylpeptides or -amino acids. Biken J. 18:105-111.
656. **Kotani, S., Y. Watanabe, T. Shimono, F. Kinoshita, T. Narita, K. Kato, D. E. S. Stewart-Tull, I. Morisaki, K. Yokogawa, and S. Kawata.** 1975. Immunoadjuvant activities of peptidoglycan subunits from the cell walls of *Staphylococcus aureus* and *Lactobacillus plantarum*. Biken J. 18:93-103.
657. **Kotani, S., I. Yanagida, K. Kato, and T. Matsuda.** 1970. Studies on peptides, glycopeptides and antigenic polysaccharide-glycopeptide complexes isolated from an L-11 enzyme lysate of the cell walls of *Mycobacterium tuberculosis* strain H37Rv. Biken J. 13:249-275.
658. **Kozloff, L. M., C. V. S. Raj, R. N. Rao, V. A. Chapman, and S. DeLong.** 1972. Structure of a transducing mycobacteriophage. J. Virol. 9:390-393.
659. **Krahenbuhl, J. L., and J. S. Remington.** 1971. In vitro induction of nonspecific resistance in macrophages by specifically sensitized lymphocytes. Infect. Immun. 4:337-343.
660. **Kraiss, J. P., S. M. Gelbart, and S. E. Juhasz.** 1973. A comparison of three mycobacteriophages. J. Gen. Virol. 20:75-87.
661. **Krasilnikov, N. A.** 1934. History of the development of soil mycobacteria (in Russian and German). Dokl. Akad. Nauk SSSR 2:577-583.
662. **Krassilnikov, N. A.** 1959. Diagnostik der Bakterien und Actinomyceten, p. 202-208. Gustav Fischer, Jena.
663. **Krassilnikov, N. A., T. V. Koronelli, B. V. Rozinov, and T. V. Kaliuzhnaya.** 1973. Mycolic acids from coloured paraffin-oxidizing mycobacteria (in Russian). Mikrobiologiya 42:240-243.
664. **Krinsky, N. I.** 1971. Function, p. 669-716. *In* O. Isler (ed.), Carotenoids. Birkhauser, Basel.
665. **Kubica, G. P.** 1973. Differential identification of mycobacteria. VII. Key features of identification of clinically significant mycobacteria. Am. Rev. Resp. Dis. 107:9-21.
666. **Kubica, G. P., I. Baess, R. E. Gordon, P. A. Jenkins. J. B. G. Kwapinski, C. McDurmont, S. R. Pattyn, H. Saito, V. Silcox, J. L. Stanford, K. Takeya, and M. Tsukamura.** 1972. A cooperative numerical analysis of rapidly growing mycobacteria. J. Gen. Microbiol. 73:55-70.
667. **Kubica, G. P., P. E. Beam, and J. W. Palmer.** 1963. A method for the isolation of unclassified acid-fast bacilli from soil and water. Am. Rev. Resp. Dis. 88:718-720.
668. **Kubica, G. P., F. P. Dunbar, and T. H. Kim.** 1973. Response of hypersensitive mice to the footpad injection of living homologous or heterologous mycobacteria: preliminary report. Appl. Microbiol. 25:718-723.
669. **Kubica, G. P., and V. A. Silcox.** 1973. Numerical taxonomic analysis of some slowly growing mycobacteria using hypothetical median strain patterns. J. Gen. Microbiol. 74:149-158.
670. **Kubica, G. P., V. A. Silcox, and E. Hall.** 1973. Numerical taxonomy of selected slowly growing mycobacteria. J. Gen. Microbiol. 74:159-167.
671. **Kubica, G. P., V. A. Silcox, J. O. Kilburn, R. W. Smithwick, R. E. Beam, W. D. Jones, Jr., and K. D. Stottmeier.** 1970. Differential identification of mycobacteria. VI. *Mycobacterium triviale* Kubica sp. nov. Int. J. Syst. Bacteriol. 20:161-174.
672. **Kubin, M., J. Kruml, Z. Horak, J. Lukavsky, and C. Vanek.** 1966. Pulmonary and nonpulmonary disease in humans due to avian mycobacteria. I. Clinical and epidemiologic analysis of nine cases observed in Czechoslovakia. Am. Res. Respir. Dis. 94:20-30.
673. **Kubin, M., A. Lind, E. Matuskova, and M. Norlin.** 1971. Studies on the precipitinogenic relationships between different serotypes of *M. avium* and *M. intracellulare*. Acta Pathol. Microbiol. Scand. Sect. B

79:850–854.
674. **Kühner, A. L., and J. R. David.** 1976. Partial characterization of murine migration inhibitory factor (MIF). J. Immunol. 116:140–145.
675. **Kunisawa, R., and R. Y. Stanier.** 1958. Studies on the role of carotenoid pigments in a chemoheterotrophic bacterium, *Corynebacterium poinsettiae*. Arch. Mikrobiol. 31:146–156.
675a. **Kunkel, H. G., and E. M. Tan.** 1964. Autoantibodies and disease, p. 351–395. *In* F. J. Dixon, Jr., and J. H. Humphrey (ed.), Advances in immunology, vol. 4. Academic Press Inc., New York.
676. **Küster, E.** 1905. Ueber Kaltblütertuberkulose. Müench. Med. Wochenschr. 52:57–59.
677. **Kusunose, E., M. Kusunose, Y. Kowa, and Y. Yamamura.** 1960. The synthesis of fatty acids in the cell-free extract of *Mycobacterium avium*. J. Biochem. (Tokyo) 47:689–693.
678. **Kuwabara, S.** 1975. Purification and properties of tuberculin-active protein from *Mycobacterium tuberculosis*. J. Biol. Chem. 250:2556–2562.
679. **Kuwabara, S.** 1975. Amino acid sequence of tuberculin-active protein from *Mycobacterium tuberculosis*. J. Biol. Chem. 250:2563–2568.
680. **Kwapinski, J. B. H., A. Alcasid, E. H. Kwapinski, and V. Nairn.** 1972. The immunology of cytoplasmic antigens of mycobacteria. Can. J. Microbiol. 18:1201–1211.
681. **LaBelle, Y.-S. L., and R. W. Walker.** 1972. The phospholipids of *Mycobacterium smegmatis*. Am. Rev. Resp. Dis. 105:625–628.
682. **Lahav, M., T. H. Chiu, and W. J. Lennarz.** 1969. Studies on the biosynthesis of mannan in *Micrococcus lysodeikticus*. II. The enzymatic synthesis of mannosyl-1-phosphoryl-undecaprenol. J. Biol. Chem. 244:5890–5898.
683. **Lakshminarayan, S., and S. Sahn.** 1973. Disseminated infection caused by *Mycobacterium avium*. Report of a case with associated leukopenia. Am. Rev. Resp. Dis. 108:123–126.
684. **Lamanna, C.** 1946. The nature of the acid-fast stain. J. Bacteriol. 52:99–103.
685. **Lampidis, T., and L. Barksdale.** 1971. Park-Williams number 8 strain of *Corynebacterium diphtheriae*. J. Bacteriol. 105:77–85.
686. **Lancet, The (Editorial).** 1972. B. C. G. vaccination. Lancet 2: 168–169.
687. **Lane, D.** 1964. *Mycobacterium ulcerans* infection in Queensland. Med. J. Austr. 1:124–125.
687a. **Lanéelle, G., and J. Asselineau.** 1962. Isolement de peptidolipides à partir de *Mycobacterium paratuberculosis*. Biochim. Biophys. Acta 59:731–732.
688. **Lanéelle, G., and J. Asselineau.** 1968. Structure d'un glycoside de peptidolipide isolé d'une mycobactérie. Eur. J. Biochem. 5:487–491.
689. **Lanéelle, G., J. Asselineau, and G. Chamoiseau.** 1971. Présence de mycosides C′ (formes simplifiées de mycoside C) dans les bacteries isolées de bovins atteints du farcin. FEBS Lett. 19:109–111.
689a. **Lanéelle, G., J. Asselineau, W. A. Wolstenholme and E. Lederer.** 1965. Determination of amino acid sequences in oligopeptides by mass spectrometry. III. Structure of a lipopeptide from *Mycobacterium johnei*. Bull. Soc. Chim. Fr., p. 2133–2134.
690. **Lanéelle, M-A., J. Asselineau, and G. Castelnuovo.** 1965. Études sur les mycobactéries et les nocardiae. IV. Composition des lipides de *Mycobacterium rhodocrous, M. pellegrino* sp., et de quelques souches de nocardiae. Ann. Inst. Pasteur Paris 108:69–82.
691. **Lanéelle, M-A., and G. Lanéelle.** 1970. Structure d'acides mycoliques et d'un intermediare dans la biosynthèse d'acides mycoliques dicarboxyliques. Eur. J. Biochem. 12:296–300.
692. **Langerova, M., and Z. Zavadilova.** 1966. Incidence of *Mycobacterium bovis* in tuberculosis patients of the Kolin county. Am. Rev. Respir. Dis. 93:136.
693. **L'Archeveque, A., P. Dionne, and J.-M. Klein.** 1970. Un cas d'adenite sous-maxillare et preauriculaire gauche a mycobacterie de Battey (*M. intracellulare*). Union Med. Can. 99:1625–1629.
694. **Lartigue, D. J., and G. L. Fite.** 1962. The chemistry of the acid-fast reaction. J. Histochem. Cytochem. 10:611–618.
695. **Lautrop, H.** 1950. Studies on antigenic structure of *Corynebacteria diphtheriae*. Acta Pathol. Microbiol. Scand. 27:443–447.
696. **Lavrin, D. H., S. A. Rosenberg, R. J. Connor, and W. D. Terry.** 1973. Immunoprophylaxis of methylcholanthrene-induced tumors in mice with *Bacillus Calmette-Guérin* and methanol-extracted residue. Cancer Res. 33:472–477.
697. **Law, J. H.** 1971. Biosynthesis of cyclopropane rings. Acc. Chem. Res. 4:199–203.
697a. **Lebar, R., J-M. Boutry, C. Vincent, R. Robineau, and G. A. Voisin.** 1976. Studies on autoimmune encephalomyelitis in the guinea pig. II. An in vitro investigation on the nature, properties, and specificity of the serum-demyelinating factor. J. Immunol. 116:1439–1446.
698. **Lechevalier, M. P., A. C. Horan, and H. Lechevalier.** 1971. Lipid composition in the classification of nocardiae and mycobacteria. J. Bacteriol. 105:313–318.
699. **Lechevalier, M. P., H. Lechevalier, and A. C. Horan.** 1973. Chemical characteristics and classification of Nocardiae. Can. J. Microbiol. 19:965–972.
700. **Lederer, E.** 1964. The origin and function of some methyl groups in branched-chain fatty acids, plant sterols and quinones. Biochem. J. 93:449–468.

701. Lederer, E. 1965. C-methylations in biological systems. Isr. J. Med. Sci. 1:1129–1147.
702. Lederer, E. 1967. Glycolipids of mycobacteria and related microorganisms. Chem. Phys. Lipids 1:294–315.
703. Lederer, E. 1969. Some problems concerning biological C-alkylation reactions and phytosterol biosynthesis. Q. Rev. 23:453–481.
704. Lederer, E. 1971. The mycobacterial cell wall. Pure Appl. Chem. 25:135–165.
705. Lederer, E., A. Adam, R. Ciorbaru, J. F. Petit, and J. Wietzerbin. 1975. Cell walls of mycobacteria and related organisms; chemistry and immunostimulant properties. Mol. Cell. Biochem. 7:87–104.
706. Lederer, E., and J. Pudles. 1951. Sur l'isolement et la constitution chimique d'un hydroxy-acide ramifié du bacille diphtérique. Bull. Soc. Chim. Biol. 33:1003–1011.
707. Lee, Y. C. 1965. Isolation and characterization of lipopolysaccharides containing 6-*O*-methyl-D-glucose from *Mycobacterium* species. J. Biol. Chem. 241:1899–1908.
708. Lee, Y. C., and C. E. Ballou. 1964. 6-*O*-methyl-D-glucose from mycobacteria. J. Biol. Chem. 239:3602–3603.
709. Lee, Y. C., and C. E. Ballou. 1964. Structural studies on the myo-inositol mannosides from the glycolipids of *Mycobacterium tuberculosis* and *Mycobacterium phlei*. J. Biol. Chem. 239:1316–1327.
710. Lee, Y. C., and C. E. Ballou. 1965. Complete structure of the glycophospholipids of mycobacteria. Biochemistry 4:1395–1404.
711. Lefford, M. J. 1975. Delayed hypersensitivity and immunity in tuberculosis. Am. Rev. Respir. Dis. 111:243–246.
712. Lehmann, K. B., and R. Neumann. 1896. Lehmann's Medezin. Handatlanten. Band X. Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bacteriologischen Diagnostik, Teil II. München.
713. Lehmann, K. B., and R. O. Neumann. 1899. Lehmann's Medezin. Handatlanten. Band X. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik, Teil II. 2. Auflage. München.
714. Lenfant, M., H. Audier, and E. Lederer. 1966. Sur une migration d'hydrogène au cours de la biosynthèse de l'acide tuberculostéarique. Bull. Soc. Chim. Fr., p. 2775–2777.
715. Lennarz, W. J., G. Scheuerbrandt, and K. Bloch, with a note by R. Ryhage. 1962. The biosynthesis of oleic and 10-methylstearic acids in *Mycobacterium phlei*. J. Biol. Chem. 237:664–671.
716. Leslie, I. W., K. Magnus, and C. J. Stewart. 1972. The prevalence of bovine type tuberculous infection in the English rural population. Tubercle 53:198–204.
717. Lessel, E. F. (Editorial Secretary for the Judicial Commission of the International Committee on Systematic Bacteriology). 1973. Opinion 47. Conservation of the specific epithet *avium* in the scientific name of the agent of avian tuberculosis. Int. J. Syst. Bacteriol. 23:472.
718. Lester, C. F., and R. J. Atwell. 1958. The tuberculin skin test reaction in active pulmonary tuberculosis. Am. Rev. Tuberc. Pulmon. Dis. 78:399–402.
719. Lester, W. 1966. Unclassified mycobacterial diseases. Annu. Rev. Med. 17:351–360.
720. Levenson, D. S., C. H. Harrison. 1966. *Mycobacterium fortuitum* corneal ulcer. Arch. Ophthalmol. 75:189–191.
721. Lewis, P., and D. Loomis. 1924. Allergic irritability. The formation of anti-sheep hemolytic amboceptor in the normal and tuberculous guinea pig. J. Exp. Med. 40:503–515.
722. Lewis, P., and D. Loomis. 1925. Allergic irratibility. II. Anaphylaxis in the guinea pig as affected by the inheritance. J. Exp. Med. 41:327–335.
723. Liaaen-Jensen, S. 1971. Isolation, reactions, p. 61–188. *In* O. Isler (ed.), Carotenoids. Birkhäuser, Basel.
724. Liaaen-Jensen, S., G. Cohen-Bazire, and R. Y. Stanier. 1961. Biosynthesis of carotenoids in purple bacteria: a reevaluation based on considerations of chemical structure. Nature (London) 192:1168–1172.
725. Lillie, R. D. 1969. H. J. Conn's biological stains, 8th ed. The Williams & Wilkins Co., Baltimore.
726. Lincoln, E. M., and L. A. Gilbert. 1972. Disease in children due to mycobacteria other than *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 105:683–714.
727. Lind, A., M. Norlin, and O. Ouchterlony. 1970. Studies on the precipitonogenic pattern of mycobacteria cultivated at various temperatures. Pneumonologie 142:283–289.
728. Linell, L., and A. Norden. 1952. Hudinfektioner i simhall genom ny art av-*Mycobacterium*. Nord. Med. 47:888–891.
729. Linell, F., and A. Norden. 1954. *Mycobacterium balnei*, a new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberc. Scand. Suppl. 33:1–85.
730. Ling, W. R. 1968. The mixed lymphocyte reaction, p. 117–145. Lymphocyte stimulation. North-Holland Publishing Co., Amsterdam; John Wiley & Sons, Inc., New York.
731. Liu, T.-Y., and E. Gotschlich. 1967. Muramic acid phosphate as a component of the mucopeptide of gram-positive bacteria. J. Biol. Chem. 242:471–476.
732. Long, E. R., and L. I. Finner. 1927. The relation of glycerol in culture media to the growth and chemical composition of tubercle bacilli. Am. Rev. Tuberc. 16:523–529.
733. Lorian, V. 1969. Direct cord reading agar in routine mycobacteriology. Appl. Microbiol. 17:559–562.
734. Lornitzo, F. A. 1972. A protein-bound phosphorylated product as an intermediate in the biosynthesis of *O*-methyl glycerol by

Mycobacterium tuberculosis. Biochim. Biophys. Acta 268:733–740.

735. **Lornitzo, F. A., and D. S. Goldman.** 1968. Intracellular localization of a 6-*O*-methyl-D-glucose containing soluble polysaccharide from *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 158:329–335.
736. **Lotte, A.** 1972. Tuberculosis in children: a cooperative study in Europe. W.H.O. Chronicle 26:550–554.
737. **Lunn, H. F.** 1965. Effects of climate on the localization and geographic distribution of skin ulcers. Dermatol. Intern. 4:111–114.
738. **Lurie, M. B.** 1936. On the mechanism of immunity in tuberculosis. The host-parasite relationship under the conditions of a localized agar focus of infection and the generalization of the disease in normal and immunized rabbits. J. Exp. Med. 63:923–946.
739. **Lurie, M. B.** 1939. Studies on the mechanism of immunity in tuberculosis. The rôle of extracellular factors and local immunity in the fixation and inhibition of growth of tubercle bacilli. J. Exp. Med. 69:555–578.
740. **Lurie, M. B.** 1964. Resistance to tuberculosis: experimental studies in native and acquired defense mechanisms. Harvard University Press, Cambridge, Mass.
741. **Lurie, M. B., and A. M. Dannenberg, Jr.** 1965. Macrophage function in infectious disease with inbred rabbits. Bacteriol. Rev. 29:466–476.
742. **Lwoff, A.** 1953. Lysogeny. Bacteriol. Rev. 17:269–337.
743. **Lynen, F.** 1961. Biosynthesis of saturated fatty acids. Fed. Proc. 20:941–951.
744. **Lynen, F.** 1967. The role of biotin-dependent carboxylations in biosynthetic reactions. Biochem. J. 102:381–400.
745. **Maberry, J. D., J. F. Mullins, and O. J. Stone.** 1965. Cutaneous infection due to *Mycobacterium kansasii*. J. Am. Med. Assoc. 194:1135–1137.
746. **MacCallum, P., J. C. Tolhurst, G. Buckle, and H. A. Sissons.** 1948. A new mycobacterial infection in man. J. Pathol. Bacteriol. 60:93–122.
747. **Macham, L. P., and C. Ratledge.** 1975. A new group of watersoluble iron-binding compounds from mycobacteria: the exochelins. J. Gen. Microbiol. 89:379–382.
748. **Macham, L. P., C. Ratledge, and J. C. Nocton.** 1975. Extracellular iron acquisition by mycobacteria: role of the exochelins and evidence against the participation of mycobactin. Infect. Immun. 12:1242–1251.
749. **Machida, Y., R. Bergeron, P. Flick, and K. Bloch.** 1973. Effects of cyclodextrins on fatty acid synthesis. J. Biol. Chem. 248:6246–6247.
750. **Machida, Y., and K. Bloch.** 1973. Complex formation between mycobacterial polysaccharides and fatty acyl-CoA derivatives. Proc. Natl. Acad. Sci. U.S.A. 70:1146–1148.
751. **Mackaness, G. B.** 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. 129:973–992.
752. **Mackaness, G. B.** 1971. Delayed hypersensitivity and the mechanism of cellular resistance to infection, p. 413–424. *In* B. Amos (ed.), Progress in immunology. International Congress of Immunology, 1st. Academic Press Inc., New York.
753. **MacLennan, I. C. M.** 1972. Antibody in the induction and inhibition of lymphocyte cytotoxicity. Transplant. Rev. 13:67–90.
754. **Maffucci, A.** 1892. Die Hühnertuberculose. Experimentelle Untersuchungen. Z. Hyg. Infektionskr. 11:445–486.
755. **Magnus, K.** 1966. Epidemiological bases of tuberculosis eradication. III. Risk of pulmonary tuberculosis after human and bovine infection. Bull. W.H.O. 35:483–508.
756. **Magnusson, M.** 1961. Specificity of mycobacterial sensitins. I. Studies in guinea pigs with purified "tuberculin" prepared from mammalian and avian tubercle bacilli, *Mycobacterium balnei*, and other acid-fast bacilli. Am. Rev. Resp. Dis. 83:57–68.
757. **Magnusson, M.** 1967. Identification of species of mycobacterium on the basis of the specificity of the delayed type reaction in guinea-pigs. Z. Tuberk. Erk. Thoraxorgane. 127: 55–56.
758. **Magnusson, M.** 1971. A comparative study of *Mycobacterium gastri* and *Mycobacterium kansasii* by delayed type skin reactions in guinea pigs. Am. Rev. Respir. Dis. 104:377–384.
759. **Magnusson, M., H. C. Engbaek, and M. Weis Bentzon.** 1961. Specificity of mycobacterial sensitins. II. Studies in guinea pigs with purified sensitin prepared from unclassified acidfast bacilli. Am. Rev. Respir. Dis. 83: 69–84.
760. **Magnusson, M., and F. Mariat.** 1968. Delineation of *Nocardia farcinica* by delayed type skin reactions on guinea pigs. J. Gen. Microbiol. 51:151–158.
761. **Maguire, A.** 1968. Lupus murinus. The discovery, diagnosis and treatment of seventeen cases of lupus murinus. Br. J. Dermatol. 80:419–423.
762. **Maië, S.** 1922. Experimentelle Versuche bei Goldfischen (*Carassius auratus*) mit säuerfesten Bazillen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Abt. 1 Orig. 88:28–38.
763. **Maillard, J., and B. R. Bloom.** 1972. Immunological adjuvants and the mechanism of cell cooperation. J. Exp. Med. 136:185–190.
764. **Majerus, P. W., and P. R. Vagelos.** 1967. Fatty acid biosynthesis and the role of the acyl carrier protein. Adv. Lipid Res. 5:1–33.
765. **Mäkelä, P. H., V. V. Valtonen, and G. Valtonen.** 1973. Role of O-antigen (lipopolysaccharide) factors in the virulence of *Salmonella*, p. 73–77. *In* E. H. Kass and S. M. Wolff (ed.), Bacterial lipopolysaccharides.

The University of Chicago Press, Chicago.
766. Mande, R. 1968. BCG vaccination, p. 140–158. Dawsons of Pall Mall, London.
767. Mankiewicz, E. 1972. Bacteriophage types of mycobacteria. Can. J. Public Health 63:342–354.
768. Manten, A. 1957. Antimicrobial susceptibility and some other properties of photochromogenic mycobacteria associated with pulmonary disease. Antonie van Leeuwenhoek; J. Microbiol. Serol. 23:357–363.
769. Marchoux, E., and F. Sorel. 1912. Recherches sur la lépre. La lépre des rats (lepramurium). Ann. Inst. Pasteur Paris 26:675–700.
770. Marchoux, M. E. 1922. La lèpre du rat est peut-être transmissible à l'homme. Bull. Acad. Med. (Paris) 87:545–547.
771. Marks, J. 1964. Aspects of the epidemiology of infection by "anonymous" mycobacteria. Proc. R. Soc. Med. 57:479–480.
772. Marks, J., and K. J. Birn. 1963. Infection due to *Mycobacterium avium*. Br. Med. J. 2:1503–1506.
773. Marks, J., and P. A. Jenkins. 1971. The opportunist mycobacteria—a 20-year retrospect. Postgrad. Med. J. 47:705–709.
774. Marks, J., P. A. Jenkins, G. S. Kilpatrick, H. C. Engbaek, and B. Vergmann. 1971. Pulmonary tuberculosis due to B.C.G. Br. Med. J. 3:229–230.
775. Marks, J., P. A. Jenkins, and W. B. Schaefer. 1969. Identification and incidence of a third type of *Mycobacterium avium*. Tubercle 50:394–395.
776. Marks, J., P. A. Jenkins, and M. Tsukamura. 1972. *Mycobacterium szulgai* - a new pathogen. Tubercle 53:210–214.
777. Marks, J., and M. Richards. 1962. Classification of the anonymous mycobacteria as a guide to their significance. (A preliminary study). Mon. Bull. Minist. Health Public Health Lab. Serv. 21:200–208.
778. Marks, J., and H. Schwabacher. 1965. Infection due to *Mycobacterium xenopei*. Br. Med. J. 1:32–33.
779. Marx, J. J., Jr., and R. Burrell. 1973. Delayed hypersensitivity to beryllium compounds. J. Immunol. 111:590–598.
780. Mascher, W. 1951. Tuberculin-negative tuberculosis. Am. Rev. Tuberc. 63:501–525.
781. Mastrangelo, M. J., Y. H. Kim, R. S. Bornstein, D. O. Chee, H. L. Sulit, J. W. Yarbro, and R. T. Prehn. 1974. Clinical and histologic correlation of melanoma regression after intralesional BCG therapy: a case report. J. Natl. Cancer Inst. 52:19–24.
782. Mathews, M. M. 1963. Studies on the localization, function and formation of the carotenoid pigments of a strain of *Mycobacterium marinum*. Photochem. Photobiol. 2:1–8.
783. Mathews, M. M. 1964. Protective effect of β-carotene against lethal photosensitization by haematoporphyrin. Nature (London) 203:1092.
784. Mathews, M. M. 1966. Ultrastructure of non-photosynthetic carotenoid-containing bacteria. J. Bacteriol. 91:1369–1371.
785. Mathews, M. M., and N. I. Krinsky. 1965. The relationship between carotenoid pigments and resistance to radiation in non-photosynthetic bacteria. Photochem. Photobiol. 4:813–817.
786. Mathews, M. M., and W. R. Sistrom. 1959. Function of carotenoid pigments in non-photosynthetic bacteria. Nature (London) 184:1892–1893.
787. Mathews, M. M., and W. R. Sistrom. 1959. Intracellular location of carotenoid pigments and some respiratory enzymes in *Sarcina lutea*. J. Bacteriol. 78:778–787.
788. Mathews, M. M., and W. R. Sistrom. 1960. The function of the carotenoid pigments of *Sarcina lutea*. Arch. Mikrobiol. 35:139–146.
789. Mathews-Roth, M. M., and N. I. Krinsky. 1970. Carotenoid pigments and the stability of the cell membrane of *Sarcina lutea*. Biochim. Biophys. Acta 203:357–359.
790. Mathews-Roth, M. M., and N. I. Krinsky. 1970. Studies on the protective function of the carotenoid pigments of *Sarcina lutea*. Photochem. Photobiol. 11:419–428.
791. Mathews-Roth, M. M., M. A. Pathak, T. B. Fitzpatrick, L. C. Harber, and E. H. Kass. 1974. β-Carotene as an oral photoprotective agent in erythropoietic protoporphyria. J. Am. Med. Assoc. 228:1004–1008.
792. Mathews-Roth, M. M., T. Wilson, E. Fujimori, and N. Krinksy. 1974. Carotenoid chromophore length and protection against photosensitization. Photochem. Photobiol. 19:217–222.
793. Mathis, P., and J. Kleo. 1973. The triplet state of β-carotene and of analog polyenes of different lengths. Photochem. Photobiol. 18:343–346.
794. Matsuhashi, M., C. P. Dietrich, and J. L. Strominger. 1965. Incorporation of glycine into the cell wall glycopeptide in *Staphylococcus aureus:* role of sRNA and lipid intermediates. Proc. Natl. Acad. Sci. U.S.A. 54:587–594.
795. Matsumura, S. 1970. Conformation of acyl carrier protein from *Mycobacterium phlei*. Biochem. Biophys. Res. Commun. 38:238–243.
796. Matsumura, S., D. N. Brindley, and K. Bloch. 1970. Acyl carrier protein from *Mycobacterium phlei*. Biochem. Biophys. Res. Commun. 38:369–377.
797. Maugh, T. H., II. 1974. Bioregulators: alteration of gene expression in citrus fruit. Science 184:655.
798. Maurice, M. T., M. J. Vacheron, and G. Michel. 1971. Isolément d'acides nocardiques de plusieurs expèces de *Nocardia*. Chem. Phys. Lipids 7:9–18.
799. Mayer, H., and O. Isler. 1971. Total synthesis, p. 325–575. *In* O. Isler (ed.), Carotenoids. Birkhauser, Basel.
800. McCarthy, C. 1971. Electronic counting in

growth studies of *Mycobacterium avium*. Appl. Microbiol. 22:546–551.
801. **McCarthy, C.** 1971. Utilization of palmitic acid by *Mycobacterium avium*. Infect. Immun. 4:199–204.
802. **McCarthy, C.** 1974. Effect of palmitic acid utilization on cell division of *Mycobacterium avium*. Infect. Immun. 9:363–372.
802a. **McCluskey, R. T., and S. Cohen (ed.).** 1974. Mechanisms of cell-mediated immunity. John Wiley & Sons, New York.
802b. **McCullough, W. G., and R. S. Merkal.** 1976. Iron-chelating compound from *Mycobacterium avium*. J. Bacteriol. 128:15–20.
803. **McCracken, G. H., and R. C. Reynolds.** 1970. Primary lymphopenic immunologic deficiency. Am. J. Dis. Child. 120:143–147.
804. **McDermott, J. C. B., A. Ben-Aziz, R. K. Singh, G. Britton, and T. W. Goodwin.** 1973. Recent studies of carotenoid biosynthesis in bacteria. Pure Appl. Chem. 35:29–45.
805. **McDonald, P. J., A. A. Tomasovic, and C. Evans.** 1971. *Mycobacterium xenopei* pulmonary infection in man. Med. J. Austr. 1:873.
806. **McMillen, S., and S. Kushner.** 1959. *Mycobacterium marinum* Aronson (1926). Bacteriol. Proc., p. 31.
807. **McNulty, M. S., and F. G. A. Winder.** 1971. Partial purification and properties of a DNA polymerase from *Mycobacterium smegmatis*. Biochim. Biophys. Acta 254:213–225.
808. **Meltzer, M. S., and E. J. Leonard.** 1973. Enhanced tumor growth in animals protected with complete Freund's adjuvant. J. Natl. Cancer. Inst. 50:209–218.
809. **Menezes, J.** 1971. Fluorescent staining of a mycobacteriophage (C2) nucleic acid. Can. J. Microbiol. 17:171–174.
810. **Menezes, J., and V. Pavilanis.** 1969. Properties of mycobacteriophage C2. Experientia 25:1112–1113.
811. **Merser, C., P. Sinaÿ and A. Adam.** 1975. Total synthesis and adjuvant activity of bacterial peptidoglycan derivatives. Biochem. Biophys. Res. Commun. 66:1316–1322.
812. **Meyer, T. J., E. F. Ribi, I. Azuma, and B. Zbar.** 1974. Biologically active components from mycobacterial cell walls. II. Suppression and regression of strain-2 guinea pig hepatoma. J. Natl. Cancer Inst. 52:103–111.
813. **Michaelis, L., and S. Granich.** 1945. Metachromasy of basic dyestuffs. J. Am. Chem. Soc. 67:1212–1219.
814. **Michel, G.** 1957. Composition des circes A de *Mycobacterium marianum*. C. R. Acad. Sci. (Paris) 244:2429–2432.
815. **Middlebrook, G.** 1956. Problèmes diagnostiques et biologiques concernant les bacilles de Koch isoniazido-résistants. Bull. Int. Union Tuberc. 26:185–213.
816. **Middlebrook, G.** 1965. The mycobacteria, p. 409–529. *In* R. J. Dubos and J. G. Hirsch (ed.), Bacterial and mycotic infections of man, 4th ed. Lippincott, Philadelphia.
817. **Middlebrook, G., C. M. Coleman, and W. B. Schaefer.** 1959. Sulfolipid from virulent tubercle bacilli. Proc. Natl. Acad. Sci. U.S.A. 45:1801–1804.
818. **Middlebrook, G., and R. J. Dubos.** 1948. Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. J. Exp. Med. 88:521–528.
819. **Middlebrook, G., R. J. Dubos, and C. Pierce.** 1947. Virulence and morphological characteristics of mammalian tubercle bacilli. J. Exp. Med. 86:175–184.
820. **Miki, K., and G. B. Mackaness.** 1964. The passive transfer of acquired resistance to *Listeria monocytogenes*. J. Exp. Med. 120:93–103.
821. **Millman, I.** 1958. Formation of protoplasts from mycobacteria by mycobacteriophage. Proc. Soc. Exp. Biol. Med. 99:216–219.
822. **Minden, P., T. R. Sharpton, and J. K. McClatchy.** 1976. Shared antigens between human malignant melanoma cells and *Mycobacterium bovis* (BCG). J. Immunol. 116:1407–1414.
823. **Minett, F. C.** 1932. Avian tuberculosis in cattle of Great Britain. J. Comp. Pathol. Ther. 45:317–330.
824. **Minnikin, D. E., H. Abdolrahimzadeh, and J. Baddiley.** 1974. The occurrence of phosphatidylethanolamine and glycosyl diglycerides in thermophilic bacilli. J. Gen. Microbiol. 83:415–418.
825. **Misaki, A., N. Ikawa, T. Kato, and S. Kotani.** 1970. Cell wall arabinogalactan of *Mycobacterium phlei*. Biochim. Biophys. Acta 215:405–408.
826. **Misaki, A., N. Seto, and I. Azuma.** 1974. Structure and immunological properties of D-arabino-D-galactans isolated from cell walls of *Mycobacterium* species. J. Biochem. (Tokyo) 73:15–27.
827. **Misaki, A., and S. Yukawa.** 1966. Studies on cell walls of mycobacteria. II. Constitution of polysaccharides from BCG cell walls. J. Biochem. (Tokyo) 59:511–520.
828. **Misaki, A., S. Yukawa, K. Tsuchiya, and T. Yamasaki.** 1966. Studies on cell walls of *Mycobacteria*. 1. Chemical and biological properties of the cell walls and the mucopeptide of BCG. J. Biochem. (Tokyo) 59:388–396.
829. **Mishell, R. I., and R. W. Dutton.** 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:423–442.
830. **Mison, P., and L. Trnka.** 1972. The effect of rifampicin on DNA-dependent RNA polymerase from mycobacteria. Collect. Czech. Chem. Commun. 37:1049–1053.
831. **Mitchison, D. A.** 1964. The virulence of tubercle bacilli from patients with pulmonary tuberculosis in India and other countries. Bull. Int. Union Tuberc. 35:287–306.
832. **Mitchison, D. A.** 1970. Regional variation in

the guinea-pig virulence and other characteristics of tubercle bacilli. Pneumonologie 142:131-137.

833. **Mitchison, D. A., J. B Selkon, and J. Lloyd.** 1963. Virulence in the guinea-pig, susceptibility to hydrogen peroxide, and catalase activity of isoniazid-sensitive bacilli from South Indian and British patients. J. Pathol. Bacteriol. 86:377-386.

834. **Mitsuda, K.** 1953. On the value of a skin reaction to a suspension of leprous nodules. (English translation of 1919 Japanese article, Int. J. Lepr. 21:347-358.) Jpn. J. Dermatol. Urol. 19:697-708.

834a. **Mittag, T., P. Kornfield, A. Tormay, and C. Woo.** 1976. Detection of anti-acetylcholine receptor factors in serum and thymus from patients with myasthenia gravis. N. Engl. J. Med. 294:691-694.

835. **Mizuguchi, Y.** 1970. Synchronization of *Mycobacterium smegmatis* culture by phenetyl alcohol and nitrosoquanidine mutagenesis (in Japanese). Med. Biol. (Tokyo) 81:215-220.

836. **Mizuguchi, Y.** 1972. Segregation of unselected markers in mycobacterial recombinants. Jpn. J. Microbiol. 16:77-81.

837. **Mizuguchi, Y.** 1974. Effect of ultraviolet-sensitive mutants on gene inheritance in mycobacterial matings. J. Bacteriol. 117:914-916.

838. **Mizuguchi, Y., and M. I. Sellers.** 1970. Isolation and characterization of temperature sensitive mutants of mycobacteriophage D29, p. 65-79. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.

839. **Mizuguchi, Y., and T. Tokunaga.** 1970. Method for isolation of deoxyribonucleic acid from mycobacteria. J. Bacteriol. 104:1020-1021.

840. **Mizuguchi, Y., and T. Tokunaga.** 1971. Recombination between *Mycobacterium smegmatis* strains *Jucho* and *Lacticola*. Jpn. J. Microbiol. 15:359-366.

841. **Modolell, M., G. A. Luckenbach, M. Parant, and P. G. Munder.** 1974. The adjuvant activity of a mycobacterial water soluble adjuvant (WSA) *in vitro*. J. Immunol. 113:395-403.

842. **Moëller, A.** 1898. Mikroorganismen, die den Tuberkelbacillen verwandt sind und bei Thieren eine miliare Tuberkelkrankheit verursachen. Vorläufige mittheilung. Dtsch. Med. Wochenschr. 24:376-379.

843. **Mohelská, H.** 1964. Morphological study of mycobacterial phage $MyF_3P/59a$, p. 551-552. *In* M. Titlbach (ed.), Proceedings of the third European Regional Conference at Prague, Vol. B, Electron microscopy, 1964. Czechoslovak Academy of Sciences, Prague.

844. **Möller, G.** 1969. Induction of DNA synthesis in normal human lymphocyte cultures by antigen-antibody complexes. Clin. Exp. Immunol. 4:65-82.

845. **Möller, G.** 1974. Effect of B-cell mitogens on lymphocyte subpopulations possessing C'_3 and Fc receptors. J. Exp. Med. 139:969-982.

846. **Möller, G., O. Sjöberg, and J. Andersson.** 1973. Immunogenicity, tolerogenicity, and mitogenicity of lipopolysaccharides. J. Infect. Dis. 128:^{s}52-^{s}56.

847. **Mollohan, C. S., and M. S. Romer.** 1951. Public health significance of swimming pool granuloma. Am. J. Public Health 51:883-891.

848. **Molony, J., and K. Connolly.** 1973. Knee arthropathy secondary to *Mycobacterium scrofulaceum*. Ann. Rheum. Dis. 32:69-71.

849. **Moore, M., and J. B. Frerichs.** 1953. An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region. Report of a case with a study of the organism, *Mycobacterium abscessus*, n. sp. J. Invest. Dermatol. 20:133-169.

850. **Moore, V. L., Q. N. Myrvik, and M. Kato.** 1972. Role of cord factor (trehalose-6,6'-dimycolate) in allergic granuloma formation in rabbits. Infect. Immun. 6:5-8.

851. **Mora, E. C., and A. Eisenstark.** 1958. Production of bacteriocin-like substances by strains of *Mycobacterium*. Bacteriol. Proc., p. 81-82.

852. **Morgan, J. K., and R. Blowers.** 1964. Swimming-pool granuloma in Britain. Lancet 1:1034-1036.

853. **Morisawa, S., A. Tanaka, K. Shojima, and Y. Yamamura.** 1960. Studies on tuberculin active peptide. I. The isolation, crystallization and properties of tuberculin active peptide from tubercle bacillus. Biochim. Biophys. Acta 38:252-258.

853a. **Morozov, V. L., M. I. Kitaev, V. D. Akulov, and L. A. Toropova.** 1976. Obimmunokhimicheskoĭ prirode i biologicheskoĭ aktivnosti autoantitel, obrazuiushchikhsia v kul'ture limfotsitov krovi bol'nykh tuberkulezom legkikh. Probl. Tuberk. (21):72-77.

854. **Morris, C. A., and G. H. Grant.** 1972. Cervical adenitis caused by *Mycobacterium chelonei* (*M. abscessus*). J. Clin. Pathol. 25:1006.

855. **Morris, C. A., G. H. Grant, P. H. Everall, and A. T. M. Myres.** 1973. Tuberculoid lymphadenitis due to *Mycobacterium chelonei*. J. Clin. Pathol. 26:422-426.

856. **Morris, S. A. C., and R. E. Subden.** 1974. Effects of ultraviolet radiation on carotenoid containing and albino strains of *Neurospora crassa*. Mutation Res. 22:105-109.

857. **Motomiya, M., M. Fujimoto, H. Sato, and S. Oka.** 1968. Biological activities of phospholipids from an unclassified mycobacteria. P-6 scotochromogen. Sci. Rep. Res. Inst. Tohoku Univ. Ser. C. 15:10-13.

858. **Motomiya, M., K. Fukushi, M. Fujimoto, H. Sato, and S. Oka.** 1968. Subcellular distribution of phospholipids in an unclassified mycobacteria, P6, scotochromogen. Sci. Rep. Res. Inst. Tohoku Univ. Ser. C. 15:14-19.

859. Motomiya, M., A. Mayama, M. Fujimoto, H. Sato, and S. Oka. 1968. A study on phospholipids from an unclassified mycobacteria, P6. Sci. Rep. Res. Inst. Tohoku Univ. Ser. C. 15:1-9.
860. Moulton, R. G., T. M. Dietz, and S. Marcus. 1972. Isolation of specific and nonspecific components from purified protein derivative. Am. Rev. Respir. Dis. 106:213-218.
861. Mudd, S. H. 1973. Biochemical mechanisms in methyl group transfer, p. 297-350. *In* W. Fishman (ed.), Metabolic conjugation and metabolic hydrolysis, vol. 3. Academic Press Inc., New York.
862. Munder, P. G., and M. Modolell. 1973. Adjuvant induced formation of lysophosphatides and their role in the immune response. Int. Arch. Allergy Appl. Immunol. 45:133-135.
863. Munder, P. G., and M. Modolell. 1974. The influence of *Mycobacterium bovis* and *Corynebacterium parvum* on the phospholipid metabolism of macrophages. Recent Results Cancer Res. 47:244-250.
864. Munro, A. J., and M. J. Taussig. 1975. Two genes in the major histocompatibility complex control immune response. Nature (London) 256:103-106.
865. Murohashi, T., E. Kondo, and K. Yoshida. 1969. The role of lipids in acid-fastness of mycobacteria. Am. Rev. Respir. Dis. 99:794-798.
866. Murohashi, T., T. Tokunaga, Y. Mizuguchi, and Y. Maruyama. 1963. Phage typing of slow growing mycobacteria. Am. Rev. Respir. Dis. 88:664-669.
867. Nakamura, R. M. 1970. Transfection of *Mycobacterium smegmatis* in an acidic medium, p. 166-178. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.
867a. Nakamura, R. M., F. V. Chisari, T. S. Edgington. 1975. Laboratory tests for diagnosis of autoimmune diseases, p. 177-203. *In* M. Stefanini (ed.), Progress in clinical pathology, vol. 6. Harcourt Brace Jovanovich, Inc., New York.
868. Nakayama, Y. 1967. The electrophoretical analysis of esterase and catalase and its use in taxonomical studies of mycobacteria. Jpn. J. Microbiol. 11:95-101.
869. Narumi, K., J. M. Keller, and C. E. Ballou. 1973. Biosynthesis of a mycobacterial lipopolysaccharide: incorporation of [^{14}C]-acyl groups by whole cells *in vivo*. Biochem. J. 132:329-340.
870. Nathan, C. F., M. L. Karnovsky, and J. R. David. 1971. Alterations of macrophage functions by mediators from lymphocytes. J. Exp. Med. 133:1356-1376.
871. Nathan, C. F., H. G. Remold, and J. R. David. 1973. Characterization of a lymphocyte factor which alters macrophage functions. J. Exp. Med. 137:275-290.
872. Nayebi, M. 1970. The effect of mechanical agitation and CO_2 on the growth of the BCG, H37Ra and R1Rv strains of *Mycobacterium tuberculosis*. J. Med. Lab. Technol. 27:218-221.
873. Neiburger, R. G., G. P. Youmans, and A. S. Youmans. 1973. Relationship between tuberculin hypersensitivity and cellular immunity to infection in mice vaccinated with viable attenuated mycobacterial cells or with mycobacterial ribonucleic acid preparations. Infect. Immun. 8:42-47.
874. Nelson, D. S. 1969. Macrophages and immunity, p. 48. North-Holland Publishing Co., Amsterdam.
875. Neta, R., and S. B. Salvin. 1973. Specific depression of delayed hypersensitivity to purified proteins, with relation to production of circulating antibody. Cell. Immunol. 9:242-250.
876. Neta, R., and S. B. Salvin. 1974. Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. J. Immunol. 113:1716-1725.
877. Nicholson, D. P., and W. R. Sevier. 1971. *Mycobacterium fortuitum* as a pathogen. Am. Rev. Respir. Dis. 104:747-750.
878. Nikitina, K. A. 1967. A study of the qualitative composition of the pigments from certain saprophytic mycobacteria. Microbiology (USSR) 36:756-761.
879. Nojima, S. 1959. Studies on the chemistry of wax D of BCG. II. On the chemical structure of oligomannoinositides. J. Biochem. (Tokyo) 46:607-620.
880. Noll, H. 1956. The chemistry of cord factor, a toxic glycolipid of *M. tuberculosis*. Adv. Tuberc. Res. 10:149-183.
881. Noll, H., H. Bloch, J. Asselineau, and E. Lederer. 1956. The chemical structure of the cord factor of *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 20:299-309.
882. Nordén, Å., and F. Linell. 1951. A new type of pathogenic *Mycobacterium*. Nature (London) 168:826.
883. Nordström, G., and J. M. Grange. 1974. Bacteriophage typing of *Mycobacterium ranae* (*fortuitum*): the use of unadapted and adapted phages in the development of a typing system. Acta Pathol. Microbiol. Scand. Sect. B. 82:87-93.
884. Norlin, M., and H. Ernevad. 1966. Purification and separation of antigens in preparations of mycobacteria. Bull. Int. Union. Tuberc. 38:29-34.
885. North, R. J. 1974. Cell mediated immunity and the response to infection, p. 185-219. *In* R. T. McCluskey and S. Cohen (ed.), Mechanisms of cell-mediated immunity. John Wiley and Sons, Inc., New York.
886. Nossal, G. J. V., and G. L. Ada. 1971. Antigens, lymphoid cells, and the immune response, p. 170-195. Academic Press Inc., New York.
887. Novick, N. 1920. The incidence of bovine in-

fection in tuberculous meningitis. J. Med. Res. 41:239-246.
888. Nussenzweig, V., C. Bianco, P. Dukor, and A. Eden. 1971. Receptors for C3 on B lymphocytes: possible role in the immune response, p. 73-82. *In* B. Amos (ed.), Progress in immunology. Academic Press Inc., New York.
889. Nyka, W. 1967. Method for staining both acid-fast and chromophobic tubercle bacilli with carbolfuchsin. J. Bacteriol. 93:1458-1460.
890. Nyka, W. 1971. Influence of oxidation and reduction on the acid-fastness of mycobacteria. Infect. Immun. 4:513-515.
891. Nyka, W., and E. F. O'Neill. 1970. A new approach to the study of non-acid fast mycobacteria. Ann. N.Y. Acad. Sci. 174:862-871.
892. Offer, R. C., A. G. Karlson, and J. A. Spittell, Jr. 1971. Infection caused by *Mycobacterium fortuitum*. Mayo Clin. Proc. 46:747-750.
893. Ogawa, T., and M. Hiraki. 1969. Studies on atypical mycobacteria. VII. The transformation of the color of atypical mycobacteria. Kitasato Arch. Exp. Med. 42:15-26.
894. Oka, S., K. Fukushi, M. Fujimoto, H. Sato, and M. Motomiya. 1968. La distribution subcellulaire des phospholipides de la Mycobacterie. C. R. Soc. Biol. 162:1648-1650.
895. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. J. Immunol. 107:1682-1689.
896. Okuyama, H., T. Kankura, and S. Nojima. 1967. Positional distribution of fatty acids in phospholipids from *Mycobacteria*. J. Biochem. (Tokyo) 61:732-737.
897. Olakowski, T., and K. Mardoń. 1971. The restorative influence of repeated tuberculin testing on tuberculin sensitivity in BCG-vaccinated schoolchildren. Bull. W. H. O. 45:649-655.
898. Olitzki, A. L., C. L. Davis, W. B. Schaefer, and M. L. Cohn. 1969. Colony variants of avian-Battey group mycobacteria intracerebrally injected into mice. Pathol. Microbiol. 34:316-323.
899. Oster, G., and Y. Nishijima. 1964. Fluorescence methods in polymer science. Fortschr. Hochpolym. Forsch. 3:313-331.
900. Oütteridge, P. M., and A. W. D. Lepper. 1973. Immunosuppressive factors released by transforming lymphocytes in the delayed hypersensitivity skin response to tuberculin. Immunology 25:981-994.
901. Owen, M., A. Smith, and J. Coultras. 1963. Granulomatous lesions occurring at site of injections of vaccines and antibiotics. South. Med. J. 56:949-952.
902. Owens, D. W., and M. E. McBride. 1969. Sporotrichoid cutaneous infection with *Mycobacterium kansasii*. Arch. Dermatol. 100:54-58.
903. Palmer, C. E. 1953. Tuberculin sensitivity and contact with tuberculosis. Further evidence of nonspecific sensitivity. Am. Rev. Tuberc. 68:678-694.
904. Palmer, C. E., and M. W. Long. 1966. Effects of infection with atypical mycobacteria on BCG vaccination and tuberculosis. Am. Rev. Respir. Dis. 94:553-568.
905. Pangborn, M. C. 1968. Structure of mycobacterial phosphatides. Ann. N.Y. Acad. Sci. 154:133-139.
906. Pangborn, M. C., and J. A. McKinney. 1966. Purification of serologically active phosphoinositides of *Mycobacterium tuberculosis*. J. Lipid Res. 7:627-633.
907. Park, W. H., and C. Krumwiede, Jr. 1911. The relative importance of the bovine and human types of tubercle bacilli in the different forms of human tuberculosis. J. Med. Res. 25:313-333.
908. Parlett, R. C., and G. P. Youmans. 1958. Antigenic relationships between ninety-eight strains of mycobacteria using gel-diffusion precipitation techniques. Am. Rev. Tuberc. Pulm. Dis. 77:450-461.
909. Paterson, A. B. 1956. The incidence and causes of tuberculin reactions in non-tuberculous cattle. Adv. Tuberc. Res. 7:101-129.
910. Patterson, R. J., and G. P. Youmans. 1970. Demonstration of tissue culture of lymphocyte-mediated immunity to tuberculosis. Infect. Immun. 1:600-603.
911. Pattyn, S. R., M. T. Hermans-Boveroulle, and J. van Ermengeur. 1968. A study on slow growing chromogenic (Runyon's group II) mycobacteria. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 207:509-516.
912. Pattyn, S. R., M. Magnusson, J. L. Stanford, and J. M. Grange. 1974. A study of *Mycobacterium fortuitum* (*ranae*). J. Med. Microbiol. 7:67-76.
913. Pattyn, S. R., J. Vandepitte, F. Portaels, and A. DeMuynck. 1971. Cases of *Mycobacterium borstelense* and *M. abscessus* infection observed in Belgium. J. Med. Microbiol. 4:145-149.
914. Pawlowski, Z., and M. G. Schultz. 1972. Taeniasis and cysticercosis (*Taenia saginata*). Adv. Parasitol. 10:269-343.
915. Pearson, C. M., B. H. Waksman, and J. T. Sharp. 1961. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. V. Changes affecting the skin and mucous membranes. Comparison of the experimental process wtih human disease. J. Exp. Med. 113:485-510.
916. Pease, A. G. E. 1968. Histochemistry, theoretical and applied, vol. 1. Little, Brown and Co., Boston.
917. Penso, G. 1953. Criteri generali per determinare la posizione sistematica di un micobatterio, p. 89-101. *In* Symposium on Actinomycetales. Morphology, Biology, and Systematics. September 7-11, 1953. VI. International Congress of Microbiology, Rome.
918. Penso, G., G. Castelnuova, A. Gaudiano, M.

Princivalle, L. Vella, and A. Zampieri. 1952. Studi e ricerche sui micobatteri. VIII. Un nouvo bacillo tubercolare: il *Mycobacterium minetti* n. sp. - Studio microbologico e patogenetico. Rend. Ist. Super. Sanita Ital. Ed. 15:491-548.

919. Perlmann, P., H. Perlmann, J. Wasserman, and T. Packalén. 1970. Lysis of chicken erythrocytes sensitized with PPD by lymphoid cells from guinea pigs immunized with tubercle bacilli. Int. Arch. Allergy Appl. Immunol. 38:204-216.
920. Perlmann, P., H. Perlmann, and H. Wigzell. 1972. Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. Transplant. Rev. 13:91-114.
921. Petit, J.-F., A. Adam, and J. Wietzerbin-Falszpan. 1970. Isolation of UDP-N-glycolylmuramyl-(Ala, Glu, DAP) from *Mycobacterium phlei*. FEBS Lett. 6:55-57.
922. Petit, J.-F., A. Adam, J. Wietzerbin-Falszpan, E. Lederer, and J. M. Ghuysen. 1969. Chemical structure of the cell wall of *Mycobacterium smegmatis*. I. Isolation and partial characterization of the peptidoglycan. Biochem. Biophys. Res. Commun. 35:478-485.
923. Petri. 1898. Zum Nachweis der Tuberkelbazillen in Butter und Milch. Arb. Gesund. 14:1-35.
924. Petroff, S. A., and W. Steenken, Jr. 1930. Biological studies of the tubercle bacillus. I. Instability of the organism-microbic dissociation. J. Exp. Med. 51:831-845.
925. Pettit, J. H. S. 1970. Infection à "*Mycobacterium ulcerans*": symptomes, diagnostic differentiel et traitment. Bull. Soc. Fr. Dermatol. Syphiligr. 77:696-699.
926. Pfuetze, K. H., and R. H. Hubble. 1968. Nontuberculous mycobacterial diseases. Dis. Mon. (Sept.), p. 1-39.
927. Pfuetze, K. H., L. V. Vo, A. F. Reimann, G. S. Berg, and W. Lester. 1965. Photochromogenic mycobacterial pulmonary disease. Am. Rev. Respir. Dis. 92:470-475.
928. Philpott, J. A., Jr., A. R. Woodburne, O. S. Philpott, W. B. Schaefer, and C. S. Mollohan. 1963. Swimming pool granuloma: a study of 290 cases. Arch. Dermatol. 88:158-162.
929. Pierard, A., and D. S. Goldman. 1963. Enzyme systems in the Mycobacteria. XIV. Fatty acid synthesis in cell-free extracts of *Mycobacterium tuberculosis*. Arch. Biochem. Biophys. 100:56-65.
929a. Pierce-Chase, C. H., R. M. Fauve, and R. Dubos. 1964. Corynebacterial pseudotuberculosis in mice. I. Comparative susceptibility of mouse strains to experimental infection with *Corynebacterium kutscheri*. J. Exp. Med. 120:267-281.
930. Pieroni, R. E., D. L. Stevens, A. Stojanovic, and L. Levine. 1972. Investigation of the responsiveness of BCG-vaccinated children with whooping-cough to tuberculin. Int. Arch. Allergy Appl. Immunol. 42:583-589.
931. Pigretti, M., E. Vilkas, E. Lederer, and H. Bloch. 1965. Propriétés chimiques et biologiques de fractions phosphatidiques isolées de "l'antigène méthylique" de *Mycobacterium tuberculosis*. Bull. Soc. Chim. Biol. 47:2039-2046.
932. Pokorny, J., E. Wisingerova, E. Sassmanova, and J. Sulova. 1968. Isolation and purification of mycobacterial phages. Am. Rev. Respir. Dis. 97:292-294.
933. Polonsky, J., G. Ferreol, R. Toubiana, and E. Lederer. 1956. Sur le "cord factor" lipide toxique de *Mycobacterium tuberculosis*: synthèses de substances à activite de "cord factor": esters de tréhalose et d'acides ramifiés synthètique. Bull. Soc. Chim. Fr., p. 1471-1478.
934. Portelance, V., and J. Asselineau. 1971. Nonphosphorous contaminants of mycobacterial phospholipid preparations. Am. Rev. Respir. Dis. 103:853-854.
935. Porter, J. W., and D. G. Anderson. 1962. The biosynthesis of carotenes. Arch. Biochem. Biophys. 97:520-528.
936. Porter, J. W., and D. G. Anderson. 1967. Biosynthesis of carotenes. Annu. Rev. Plant Physiol. 18:197-228.
937. Porter, J. W., and R. E. Lincoln. 1950. 1. *Lycopersicon* selections containing a high content of carotenes and colorless polyenes. II. The mechanism of carotene biosynthesis. Arch. Biochem. Biophys. 27:390-403.
938. Prebble, J., and A. S. Huda. 1973. Sensitivity of the electron transport chain of pigmented and nonpigmented *Sarcina* membranes to photodynamic action. Photochem. Photobiol. 17:255-264.
939. Prebble, J., J. Turner, and M. Anwar. 1976. The protective role of carotenoids in micrococci. Proc. Soc. Gen. Microbiol. 3:95.
940. Prissick, F., and A. M. Masson. 1952. A preliminary report on a study of pigmented mycobacteria. Can. J. Public Health 43:34-35.
941. Prissick, F. H., and A. M. Masson. 1956. Cervical lymphadenitis caused by chromogenic mycobacteria. Can. Med. Assoc. J. 75:798-803.
942. Prissick, F. H., and A. M. Masson. 1957. Yellow-pigmented pathogenic mycobacteria from cervical lymphadenitis. Can. J. Microbiol. 3:91-100.
943. Procházka, P., M. Nohýnek, and J. Rokos. 1972. Esterases in mycobacteria. IV. The effect of macromolecular compounds isolated from a submerged culture of *Mycobacterium phlei* on the temporary activation of esterases. Folia Microbiol. (Prague) 17:205-212.
944. Promé, J-C, R. W. Walker, and C. Lacave. 1974. Condensation de deux molécules d'acide palmitique chez *Corynebacterium*

diphtheriae: formation d'un β-céto-ester de tréhalose. C. R. Acad. Sci. Ser. C 278:1065–1068.

945. **Prove, O.** 1887. *Micrococcus ochroleucus*, eine neue chromogene Spaltpilzform. Beitr. Biol. Pflanz. 4:409–439.
946. **Průchová, J., E. Švandová, and R. Skrivanová.** 1970. Experimental study of the immunogenic and allergenic activity of different batches of Czechoslovak and Danish BCG vaccine in guinea pigs. J. Hyg. Epidemiol. Microbiol. Immunol. 14:469–480.
947. **Ptashne, M.** 1967. Isolation of the λ phage repressor. Proc. Natl. Acad. Sci. U.S.A. 57:306–313.
948. **Pudles, J., and E. Lederer.** 1953. Sur la constitution chimique d'un acide mycolique insaturé isolé du Bacille diphtérique (*Corynebacterium diphtheriae*). Biochim. Biophys. Acta 11:163–164.
949. **Pudles, J., and E. Lederer.** 1954. Sur l'isolement et la constitution chimique de l'acide coryno-mycolénique et de deux cétones des lipides du bacille diphtérique. Bull. Soc. Chim. Biol. 36:759–777.
950. **Pust, R. E., H. O. N. Onubogu, L. I. Egornu, and R. Smithwick.** 1973. Pulmonary disease due to *Mycobacterium fortuitum* in a Nigerian. Am. Rev. Respir. Dis. 108:1416–1420.
951. **Quagliata, F., and J. M. Phillips-Quagliata.** 1972. Competence of thoracic duct cells in the transfer of adjuvant disease and delayed hypersensitivity. Evidence that mycobacterial components are required for the successful transfer of the disease. Cell. Immunol. 3:78–87.
952. **Radochová, M., J. Konicek, and I. Malek,** 1966. The mutagenic effect of ultraviolet radiation and nitrous acid on *Mycobacterium phlei*. Folia Microbiol. (Prague) 11:163–168.
953. **Raffel, S.** 1948. The components of the tubercle bacillus responsible for the delayed type of "infectious" allergy. J. Infect. Dis. 82:267–293.
954. **Raffel, S.** 1955. The mechanism involved in acquired immunity to tuberculosis, p. 261–282. *In* G. E. W. Wolstenholme and M. P. Cameron (ed.), CIBA foundation symposium on experimental tuberculosis bacillus and host, with an addendum on leprosy. Little, Brown and Co., Boston.
955. **Raj, C. V. S., and T. Ramakrishnan.** 1970. Transduction in *Mycobacterium smegmatis*. Nature (London) 228:280–281.
956. **Raj, C. V. S., and T. Ramakrishnan.** 1971. Genetic studies in mycobacteria: isolation of auxotrophs and mycobacteriophages for *Mycobacterium smegmatis* and their use in transduction. J. Ind. Inst. Sci. 53:126–140.
957. **Raleigh, J. W., and R. Wichelhausen.** 1973. Exogenous reinfection with *Mycobacterium tuberculosis* confirmed by phage typing. Am. Rev. Respir. Dis. 108:639–642.
958. **Ramakrishnan, T., P. S. Murthy, and K. P. Gopinathan.** 1972. Intermediary metabolism of mycobacteria. Bacteriol. Rev. 36:65–108.
959. **Ramsey, V. G.** 1953. Protoporphyrin dimethyl ester. p. 39–43. *In* E. E. Snell (ed.), Biological preparations, vol. 3. John Wiley and Sons, Inc., New York.
960. **Ratledge, C., and B. J. Marshall.** 1972. Iron transport in *Mycobacterium smegmatis*: the role of mycobactin. Biochim. Biophys. Acta 279:58–74.
961. **Razin, S., and Z. Shafer.** 1969. Incorporation of cholesterol by membranes of bacterial L-phase variants, with an appendix: on the determination of the L-phase parentage by the electrophoretic patterns of cell proteins. J. Gen. Microbiol. 58:327–339.
962. **Redmond, W. B.** 1963. Bacteriophages of the mycobacteria: a review. Adv. Tuberc. Res. 12:191–229.
963. **Reed, G. B.** 1957. *Mycobacterium microti* Reed, nom. nov., p. 703–704. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology, ed. 7. The Williams & Wilkins Co., Baltimore.
964. **Reggiardo, Z., and G. Middlebrook.** 1974. Failure of passive serum transfer of immunity against aerogenic tuberculosis in rabbits. Proc. Soc. Exp. Biol. Med. 145:173–175.
965. **Reid, I. S.** 1967. *Mycobacterium ulcerans* infection: a report of 13 cases at the Port Moresby General Hospital, Papua. Med. J. Aust. 1:427–431.
966. **Reid, J. D.** 1970. The pathogenesis of the immunologic granuloma. N. Z. Med. J. 71:375–378.
967. **Reznikov, M., and D. J. Dawson.** 1973. Serological examination of some strains that are in the *Mycobacterium avium-intracellulare-scrofulaceum* complex but do not belong to Schaefer's serotypes. Appl. Microbiol. 26:470–473.
968. **Reznikov, M., and J. H. Leggo.** 1972. Modification of Schaefer's procedure for serotyping of organisms of the *Mycobacterium avium-M. intracellulare-M. scrofulaceum* complex. Appl. Microbiol. 23:819–823.
969. **Reznikov, M., J. H. Leggo, and D. J. Dawson.** 1971. Investigation by seroagglutination of strains of the *Mycobacterium intracellulare-M. scrofulaceum* group from house dusts and sputum in southeastern Queensland. Am. Rev. Respir. Dis. 104:951–953.
970. **Ribi, E., R. L. Anacker, W. R. Barclay, W. Brehmer, S. C. Harris, W. R. Leif, and J. Simmons.** 1971. Efficacy of mycobacterial cell walls as a vaccine against airborne tuberculosis in the Rhesus monkey. J. Infect. Dis. 123:527–538.
971. **Ribi, E., W. Brehmer, K. C. Milner, and W. C. Wicht.** 1970. Specific and nonspecific stimulation of resistance in mice against *Mycobacterium tuberculosis* H37Rv. Pneu-

monologie 142:264–273.
972. **Rich, A. R.** 1944. The pathogenesis of tuberculosis. Charles C Thomas, Publisher, Springfield, Ill.
973. **Rich, A. R.** 1951. The pathogenesis of tuberculosis, 2nd ed. Charles C Thomas, Publisher, Springfield, Ill.
974. **Rich, A. R., and M. R. Lewis.** 1932. The nature of allergy in tuberculosis as revealed by tissue culture studies. Bull. Johns Hopkins Hosp. 50:115–131.
975. **Richards, O. W.** 1941. The staining of acid-fast tubercle bacteria. Science 93:190.
976. **Richerson, H. B.** 1971. Cutaneous basophil (Jones-Mote) hypersensitivity after "tolerogenic" doses of intravenous ovalbumin in the guinea pig. J. Exp. Med. 134:630–641.
977. **Richerson, H. B., H. F. Dvorak, and S. Leskowitz.** 1969. Cutaneous basophilic hypersensitivity: a new interpretation of the Jones-Mote reaction. J. Immunol. 103:1431–1434.
978. **Richmond, L., and M. M. Cummings.** 1950. An evaluation of methods of testing the virulence of acid-fast bacilli. Am. Rev. Tuberc. 62:632–637.
979. **Richter, J., and R. Halova.** 1968. Quantitative comparison between microscopical demonstration of tubercle bacilli by the fluorescence method and the classical staining technique after Ziehl-Neelsen and with cultivation liquid and solid media. Rozhl. Tuberk. Nemocech Plicn. 28:440–444.
980. **Richter, P. E., A. A. Tomasovic, and T. G. Paxon.** 1969. Pulmonary disease related to *Mycobacterium xenopei*. Med. J. Aust. 1:1246–1247.
981. **Ridell, M., and M. Norlin.** 1973. Serological study of nocardia by using mycobacterial precipitation reference systems. J. Bacteriol. 113:1–7.
982. **Rilling, H. C.** 1962. Photoinduction of carotenoid synthesis of a *Mycobacterium* sp. Biochim. Biophys. Acta 60:548–556.
983. **Rilling, H. C.** 1964. On the mechanism of photoinduction of carotenoid synthesis. Biochim. Biophys. Acta 79:464–475.
984. **Rilling, H. C.** 1965. A study of inhibition of carotenoid synthesis. Arch. Biochem. Biophys. 110:39–46.
985. **Robbins, S. L.** 1967. Pathology, 3rd ed. W. B. Saunders Co., Philadelphia and London.
986. **Roberts, D. B., G. L. Wright, Jr., L. F. Affronti, and M. Reich.** 1972. Characterization and comparison of mycobacterial antigens by two-dimensional immunoelectrophoresis. Infect. Immun. 6:564–573.
987. **Rocklin, R. E.** 1973. Production of migration inhibitory factor by non-dividing lymphocytes. J. Immunol. 110:674–678.
987a. **Rocklin, R. E., J. L. Kitzmiller, C. B. Carpenter, M. R. Garovoy, and J. R. David.** 1976. Maternal-fetal relation: absence of an immunologic blocking factor from the serum of women with chronic abortions. N. Engl. J. Med. 295:1209–1213.
988. **Rocklin, R. E., R. P. MacDermott, L. Chess, S. F. Schlossman, and J. R. David.** 1974. Studies on mediator production by highly purified human T and B lymphocytes. J. Exp. Med. 140:1303–1316.
989. **Rodda, G. M. J.** 1964. Phage patterns of the fast-growing acid-fast bacteria. Aust. J. Exp. Biol. Med. Sci. 42:457–464.
990. **Roos, G. B., and L. Ballester.** 1969. Spheroplastes de B. C. G. induits par un bacteriophage specifique. Ann. Inst. Pasteur Lille 20:75–84.
991. **Rosenthal, S. R.** 1972. BCG in cancer and leukemia. Bull. Inst. Pasteur Paris 70:29–50.
991a. **Ross, G. W., L. Singleton, and K. V. Chanter.** 1967. Isolation of a peptidoglycolipid from *M. johnei* and its possible use in intradermal tests. J. Comp. Pathol. 77:255–262.
992. **Ross, J. A.** 1960. *Mycobacterium salmoniphilum* sp. nov. from salmonoid fishes. Am. Rev. Respir. Dis. 81:241–250.
993. **Rowley, D., K. J. Turner, and C. R. Jenkin.** 1964. The basis for immunity to mouse typhoid. III. Cell-bound antibody. Aust. J. Exp. Biol. Med. Sci. 42:237–248.
994. **Runyon, E. H.** 1959. Anonymous mycobacteria in pulmonary disease. Med. Clin. North Am. 43:273–290.
995. **Runyon, E. H.** 1965. Pathogenic mycobacteria. Adv. Tuberc. Res. 14:235–287.
996. **Runyon, E. H.** 1967. *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. 95:861–865.
997. **Runyon, E. H.** 1970. Identification of mycobacterial pathogens utilizing colony characteristics. Am. J. Clin. Pathol. 54:578–586.
998. **Runyon, E. H.** 1972. Mycobacteria and mycobacterioses. Kekkaku 47:331–337.
999. **Runyon, E. H.** 1974. Filament formation by mycobacteria, p. 39. *In* Proceedings of the First International Nocardial Conference, Merida, Venezuela. McCowan Printing Co., Augusta, Ga.
1000. **Runyon, E. H.** 1974. Rejection of *Mycobacterium aquae*: request for an opinion. Int. J. Syst. Bacteriol. 24:532–533.
1001. **Runyon, E. H.** 1974. Ten mycobacterial pathogens. Tubercle 55:235–240.
1002. **Runyon, E. H., A. G. Karlson, G. P. Kubica, and L. G. Wayne.** 1974. *Mycobacterium*, p. 148–174. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, ed. 2. American Society for Microbiology, Washington, D.C.
1003. **Russell, R. L., G. J. Jann, and S. Froman.** 1960. Lysogeny in the mycobacteria. I. The establishment of lysogeny. Am. Rev. Respir. Dis. 82:384–393.
1004. **Russell, R. L., G. J. Jann, and S. Froman.** 1963. Lysogeny in the mycobacteria. II. Some phage-host relationships of lysogenic mycobacteria. Am. Rev. Respir. Dis. 88:528–538.
1005. **Sage, R. E., and A. W. Derrington.** 1973. Op-

portunistic cutaneous *Mycobacterium marinum* infection mimicking *Mycobacterium ulcerans* in lymphosarcoma. Med. J. Aust. 2:434–437.

1006. **Saier, M. H., Jr., and C. E. Ballou.** 1968. The 6-*O*-methylglucose-containing lipopolysaccharide of *Mycobacterium phlei*. J. Biol. Chem. 243:4332–4341.

1007. **Saier, M. H., Jr., and C. E. Ballou.** 1968. The 6-*O*-methylglucose-containing lipopolysaccharide of *Mycobacterium phlei*: identification of D-glyceric acid and 3-*O*-methyl-D-glucose in the polysaccharide. J. Biol. Chem. 243:992–1005.

1008. **Saier, M. H., Jr., and C. E. Ballou.** 1968. The 6-*O*-methylglucose-containing lipopolysaccharide of *Mycobacterium phlei*: structure of the reducing end of the polysaccharide. J. Biol. Chem. 243:4319–4331.

1009. **Saito, H.** 1973. Studies on mycobacteria isolated from monkeys: description of *Mycobacterium budapestae* sp. nov., p. 193–198. *In* J. G. Weiszfeiler (ed.), Atypical mycobacteria. Akademiae Kiado, Budapest.

1010. **Saito, H., H. Tasaka, S. Osasa, T. Yamura, T. Fukuhara, and A. Yamada.** 1974. Disseminated *Mycobacterium intracellulare* infection. Am. Rev. Respir. Dis. 109:572–576.

1011. **Saito, K., M. Nakano, T. Akiyama, and D. Ushiba.** 1962. Passive transfer of immunity to typhoid by macrophages. J. Bacteriol. 84:500–507.

1012. **Saito, R., A. Tanaka, K. Sugiyama, I. Azuma, Y. Yamamura, M. Kato, and M. B. Goren.** 1976. Adjuvant effect of cord factor, a mycobacterial lipid. Infect. Immun. 13:776–781.

1013. **Saito, R., A. Tanaka, K. Sugiyama, and M. Kato.** 1975. Cord factor not toxic in rats. Am. Rev. Respir. Dis. 112:578–580.

1013a. **Salabé, G. B.** 1975. Immunochemistry of the interaction of thyroglobulin (Tg) and its auto- and hetero antibodies. Acta Endocrinol. (Copenhagen) 196 Suppl.:1–25.

1014. **Salton, M. R. J.** 1974. Membrane associated enzymes in bacteria. Adv. Microb. Physiol. 11:213–283.

1015. **Salton, M. R. J., and J. H. Freer.** 1965. Composition of the membranes isolated from several gram-positive bacteria. Biochim. Biophys. Acta 107:531–538.

1016. **Salvin, S. B., and R. Neta.** 1975. A possible relationship between delayed hypersensitivity and cell-mediated immunity. Am. Rev. Respir. Dis. 111:373–377.

1016a. **Salvin, S. B., J. Nishio, and J. T. Shonnard.** 1974. Two new inhibitory activities in blood of mice with delayed hypersensitivity, after challenge with specific antigen. Infect. Immun. 9:631–635.

1017. **Salvin, S. B., J. S. Youngner, and W. H. Lederer.** 1973. Migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. Infect. Immun. 7:68–75.

1018. **Sato, S., and M. Imi.** 1968. The surface structure of *M. leprae*. Int. J. Lepr. 36:303–308.

1019. **Schaefer, W. B.** 1965. Serologic identification and classification of the atypical mycobacteria by their agglutination. Am. Rev. Respir. Dis. 92(Suppl.):85–93.

1020. **Schaefer, W. B.** 1967. Serologic identification of the atypical mycobacteria and its value in epidemiologic studies. Am. Rev. Respir. Dis. 96:115–118.

1021. **Schaefer, W. B.** 1967. Type-specificity of atypical mycobacteria in agglutination and antibody absorption tests. Am. Rev. Respir. Dis. 96:1165–1168.

1022. **Schaefer, W. B.** 1968. Incidence of the serotypes of *Mycobacterium avium* and atypical mycobacteria in human and animal diseases. Am. Rev. Respir. Dis. 97:18–23.

1023. **Schaefer, W. B., J. V. Beer, N. A. Wood, E. Boughton, P. A. Jenkins, and J. A. Marks.** 1973. A bacteriological study of endemic tuberculosis in birds. J. Hyg. 71:549–557.

1024. **Schaefer, W. B., K. J. Birn, P. A. Jenkins, and J. Marks.** 1969. Infection with the avian-Battey group of mycobacteria in England and Wales. Br. Med. J. 2:412–415.

1025. **Schaefer, W. B., C. L. Davis, and M. L. Cohn.** 1970. Pathogenicity of transparent, opaque, and rough variants of *Mycobacterium avium* in chickens and mice. Am. Rev. Respir. Dis. 102:499–506.

1026. **Schaefer, W. B., and C. W. Lewis, Jr.** 1965. Effect of oleic acid on growth and cell structure of mycobacteria. J. Bacteriol. 90:1438–1447.

1027. **Schaefer, W. B., A. Marshak, and B. Burkhart.** 1949. The growth of *Mycobacterium tuberculosis* as a function of its nutrients. J. Bacteriol. 58:549–563.

1028. **Schaefer, W. B., E. Wolinsky, P. A. Jenkins, and J. Marks.** 1973. *Mycobacterium szulgai*—a new pathogen, serologic identification and report of five new cases. Am. Rev. Respir. Dis. 108:1320–1326.

1029. **Schinitsky, M. R., L. R. Hyman, A. A. Blazkovec, and P. M. Burkholder.** 1973. *Bacillus Calmette-Guérin* vaccination and skin tumor promotion with croton oil in mice. Cancer Res. 33:659–663.

1030. **Schleifer, K. H., and O. Kandler.** 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36:407–477.

1031. **Schmelev, N. A.** 1970. Polymorphism of mycobacteria and shifts of host immunity. Bull. Int. Union Tuberc. 43:179–181.

1032. **Schonell, M. E., J. W. Crofton, A. E. Stuart, and A. Wallace.** 1968. Disseminated infection with *Mycobacterium avium*. I. Clinical features, treatment and pathology. Tubercle 49:12–30.

1033. **Schröder, K. H., and M. Magnusson.** 1968. *Mycobacterium kansasii* und seine varianten. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 207:498–509.

1034. **Schultz, J., and A. D. Elbein.** 1974. Biosynthesis of mannosyl- and glucosyl-phosphoryl polyprenols in *Mycobacterium smegmatis:* evidence for oligosaccharide-phosphoryl-polyprenols. Arch. Biochem. Biophys. 160: 311-322.

1035. **Schultz, J. C., and A. D. Elbein.** 1974. Biosynthesis of glycosyl-diglycerides in *Mycobacterium smegmatis*. J. Bacteriol. 117:107-115.

1036. **Schwab, J. H.** 1975. Suppression of the immune response by microorganisms. Bacteriol. Rev. 39:121-143.

1037. **Schwabacher, H.** 1959. A strain of *Mycobacterium* isolated from skin lesions of a cold-blooded animal, *Xenopus laevus* and its relation to atypical acid-fast bacilli occurring in man. J. Hyg. 57:57-67.

1038. **Segal, W.** 1965. Comparative study of *in vivo* and *in vitro* grown *Mycobacterium tuberculosis*. IV. Immunogenic differentiation. Proc. Soc. Exp. Biol. Med. 118:214-218.

1039. **Segal, W.** 1965. Comparative study of mycobacterium grown in vivo and in vitro. V. Differences in staining properties. Am. Rev. Respir. Dis. 91:285-287.

1040. **Segal, W., and H. Bloch.** 1956. Biochemical differentiation of *Mycobacterium tuberculosis* grown *in vivo* and *in vitro*. J. Bacteriol. 72:132-141.

1041. **Segal, W., and H. Bloch.** 1957. Pathogenic and immunogenic differentiation of *Mycobacterium tuberculosis* grown in vitro and in vivo. Am. Rev. Tuberc. Pulm. Dis. 75:495-500.

1042. **Segal, W., and W. T. Miller.** 1965. Comparative study of in vivo and in vitro grown *Mycobacterium tuberculosis*. III. Lipid composition. Proc. Soc. Exp. Biol. Med. 118:613-616.

1043. **Seibert, F. B.** 1949. The isolation of three different proteins and two polysaccharides from tuberculin by alcohol fractionation: their chemical and biological properties. Am. Rev. Tuberc. 59:86-101.

1044. **Siebert, F. B., and J. T. Glen.** 1941. Tuberculin purified protein derivative. Preparation and analyses of large quantity for standard. Am. Rev. Tuberc. 44:9-25.

1045. **Sellers, M. I.** 1970. Changes in properties of D29 phage affected by host-range mutation, p. 39-52. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.

1046. **Sellers, M. I., and T. Tokunaga.** 1970. Inactivation of mycobacteriophages by lipid solvents, p. 134-143. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.

1047. **Sellers, M. I., K. Tokuyasu, Z. Price, and S. Froman.** 1957. Electron microscopic studies of mycobacteriophages. Am. Rev. Tuberc. Pulmon. Dis. 76:964-969.

1048. **Sever, J. L.** 1960. Passive transfer of resistance to tuberculosis through use of monocytes. Proc. Soc. Exp. Biol. Med. 103:326-329.

1049. **Shaw, N.** 1970. Bacterial glycolipids. Bacteriol. Rev. 34:365-377.

1050. **Shevach, E., R. Herberman, R. Liebermann, M. M. Frank, and I. Green.** 1972. Receptors for immunoglobulin and complement on mouse leukemias and lymphomas. J. Immunol. 108:325-328.

1051. **Shilo, M.** 1963. The infection-promoting activity of high molecular weight acidic microbial polysaccharides. Bull. Res. Counc. Isr. 11(A4):310-318.

1052. **Shima, K., A. M. Dannenberg, M. Ando, S. Chandrasekhar, J. A. Seluzicki, and J. I. Fabrikant.** 1972. Macrophage accumulation, division, maturation and digestive and microbicidal capacities in tuberculous lesions. I. Studies involving their incorporation of tritiated thymidine and their content of lysomal enzymes and bacilli. Am. J. Pathol. 67:159-180.

1053. **Shocket, E., R. E. Drosd, and C. F. Tate, Jr.** 1964. Granuloma of the skin due to *Mycobacterium fortuitum*. S. Med. J. 57:1352-1356.

1053a. **Shulman, S.** 1971. Thyroid antigens and autoimmunity, p. 85-185. *In* F. J. Dixon and H. G. Kunkel (ed.), Advances in immunology, vol. 14. Academic Press Inc., New York.

1054. **Silver, J., and B. Benacerraf.** 1974. Dissociation of T cell helper function and delayed hypersensitivity. J. Immunol. 113:1872-1875.

1055. **Simmons, R. L., A. Rios, and J. H. Kersey.** 1972. Regression of spontaneous mammary carcinomas using direct injections of neuraminidase and BCG. J. Surg. Res. 12:57-61.

1056. **Simon, C.** 1966. Infections par mycobacteries atypiqies. Pediatrie 21:33-45.

1057. **Singleton, L., G. W. Ross, and K. V. Chanter.** 1970. Comparative intradermal test with peptidoglycolipids (PMKO) from *Mycobacterium avium* and *Mycobacterium johnei*. J. Comp. Pathol. 80:31-35.

1058. **Sistrom, W. R., M. Griffiths, and R. Y. Stanier.** 1956. The biology of a photosynthetic bacterium which lacks colored carotenoids. J. Cell. Comp. Physiol. 48:473-515.

1059. **Sivcev, J., and R. Bönicke.** 1970. Host-controlled changes of phage phlei B02 by *Nocardia asteroides* and *Nocardia brasiliensis*, p. 53-64. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.

1060. **Sjöberg, O., and E. Hedfors.** 1974. Antibody-induced lymphocyte-mediated cytotoxicity in sarcoidosis patients and healthy controls. Cell. Immunol. 11:401-408.

1060a. **Sjögren, H. O., I. Hellström, S. C. Bansal, and K. E. Hellström.** 1971. Suggestive evi-

dence that the "blocking antibodies" of tumor-bearing individuals may be antigen-antibody complexes. Proc. Natl. Acad. Sci. U.S.A. 68:1372-1375.

1061. Šlosárek, M. 1970. DNA base composition and Adansonian analysis of mycobacteria. Folia Microbiol. (Prague) 15:431-436.

1062. Slotnick, I. J., and H. J. Sacks. 1973. Osteomyelitis caused by *Mycobacterium fortuitum*. Report of a case. Am. J. Clin. Pathol. 59:574-575.

1063. Smith, D. T. 1967. Diagnostic and prognostic significance of the quantitative tuberculin tests. The influence of subclinical infections with atypical mycobacteria. Ann. Intern. Med. 67:919-946.

1064. Smith, D. T., N. F. Conant, J. R. Overman, J. W. Beard, H. P. Willett, J. E. Larsch, Jr., D. B. Amos, C. M. Zmijewski, E. Glassman, S. Osterhout, and D. G. Sharp (ed.). 1964. *In* Zinsser microbiology, ed. 13. Appleton-Century-Crofts, New York.

1065. Smith, D. T., D. S. Martin, N. F. Conant, J. W. Beard, G. Taylor, H. I. Kohn, and M. A. Poston. 1948. Zinsser's textbook of bacteriology, ed. 9, p. 143. Appleton-Century-Crofts, New York.

1066. Smith, D. W., H. M. Randall, M. M. Gastambide-Odier, and A. L. Koevoet. 1957. The characterization of mycobacterial strains by the composition of their lipide extracts. Ann. N.Y. Acad. Sci. 69:145-157.

1067. Smith, D. W., H. M. Randall, A. P. MacLennan, and E. Lederer. 1960. Mycosides: a new class of type-specific glycolipids of *Mycobacteria*. Nature (London) 186:887-888.

1068. Smith, D. W., H. M. Randall, A. P. MacLennan, R. K. Putney, and S. V. Rao. 1960. Detection of specific lipids in mycobacteria by infrared spectroscopy. J. Bacteriol. 79:217-229.

1069. Smith, J. H. 1970. Epidemiologic observations on cases on Buruli ulcer seen in a hospital in the lower Congo. Am. J. Trop. Med. Hyg. 19:657-663.

1070. Smith, T. 1896. Two varieties of the tubercle bacillus from mammals. Trans. Assoc. Am. Physicians 11:75-93.

1071. Smith, W. L., and C. E. Ballou. 1973. The 6-O-methylglucose-containing lipopolysaccharides of *Mycobacterium phlei*. Locations of the neutral and acidic acryl groups. J. Biol. Chem. 248:7118-7125.

1072. Snow, G. A., and A. J. White. 1969. Chemical and biological properties of mycobactins isolated from various mycobacteria. Biochem. J. 115:1031-1045.

1073. Snyder, M. L., W. Bullock, and R. B. Parker. 1967. Studies on the oral filamentous bacteria. I. Cell wall composition of *Actinomyces, Nocardia, Bacterionema,* and *Leptotrichia*. J. Infect. Dis. 117:332-340.

1074. Sohler, A., A. H. Romano, and W. J. Nickerson. 1958. Biochemistry of the *Actinomycetales*. III. Cell wall composition and the action of lysozyme upon cells and cell walls of the *Actinomycetales*. J. Bacteriol. 75:283-290.

1075. Sommer, A. F. J., R. M. Williams, and A. D. Mandel. 1962. *Mycobacterium balnei* infection. Report of two cases. Arch. Dermatol. 86:316-323.

1076. Sorkin, E. 1963. Cytophilic antibody, p. 38-61. *In* G. E. W. Wolstenholme and J. Knight (ed.), The immunologically competent cell: its nature and origin. Ciba Foundation Study Group no. 16. Little, Brown and Co., Boston.

1077. Sowell, J. M., R. M. Russell, and V. J. Ionata. 1973. Tuberculin tine test conversion among United States Army enlisted personnel in Vietnam. Milit. Med. 138:96-98.

1078. Spector, W. G. 1969. The granulomatous inflammatory exudate. Int. Rev. Exp. Pathol. 8:1-55.

1079. Spitznagel, J., and A. C. Allison. 1970. Mode of action of adjuvants: effects of antibody responses to macrophage-associated bovine serum albumin. J. Immunol. 104:128-139.

1080. Stadecker, M. J., and S. Leskowitz. 1974. The cutaneous basophil response to mitogens. J. Immunol. 113:496-500.

1081. Stanford, J. L. 1973. An immunodiffusion analysis of strains of *Mycobacterium ulcerans* isolated in Australia, Malaya, Mexico, Uganda and Zaire. J. Med. Microbiol. 6:405-408.

1082. Stanford, J. L. 1973. An immunodiffusion analysis of *Mycobacterium lepraemurium* Marchoux and Sorel. J. Med. Microbiol. 6:435-439.

1083. Stanford, J. L., and A. Beck. 1968. An antigenic analysis of the mycobacteria. *Mycobacterium fortuitum, Myco. kansasii, Myco. phlei, Myco. smegmatis* and *Myco. tuberculosis*. J. Pathol. Bacteriol. 95:131-139.

1084. Stanford, J. L., and A. Beck. 1969. Bacteriological and serological studies of fast growing mycobacteria identified as *Mycobacterium friedmannii*. J. Gen. Microbiol. 58:99-106.

1085. Stanford, J. L., and W. J. Gunthorpe. 1969. Serological and bacteriological investigation of *Mycobacterium ranae* (*fortuitum*). J. Bacteriol. 98:375-383.

1086. Stanford, J. L., and W. J. Gunthorpe. 1971. A study of some fast-growing scotochromogenic mycobacteria including species descriptions of *Mycobacterium gilvum* (new species) and *Mycobacterium duvalii* (new species). Br. J. Exp. Pathol. 52:627-637.

1087. Stanford, J. L., S. R. Pattyn, F. Portaels, and W. J. Gunthorpe. 1972. Studies on *Mycobacterium chelonei*. J. Med. Microbiol. 5:177-182.

1088. Stanford, J. L., W. D. L. Revill, W. J. Gunthorpe, and J. M. Grange. 1975. The production and preliminary investigation of burulin, a new skin test reagent for *Mycobacterium ulcerans* infection. J. Hyg. 74:7-16.

1089. **Stanier, R. Y.** 1959. Formation and function of the photosynthetic pigment system in purple bacteria, p. 43-53. *In* The photochemical apparatus, its structure and function. Rep. Symp. June 16-18, 1958, Biol. Dept., Brookhaven National Laboratory, Upton, N.Y.

1090. **Stanier, R.** 1960. Carotenoid pigments: problems of synthesis and function. Harvey Lect. 1958-1959, Ser. 54, p. 219-255.

1091. **Stead, W. W., and J. H. Bates.** 1971. Contribution of phage typing of *M. tuberculosis* in the study of the pathogenesis of chronic tuberculosis in man. Trans. 30th VA-Armed Forces Pulm. Dis. Res. Conf., p. 15-16.

1092. **Steenken, Jr., W.** 1935. Lysis of the tubercle bacillus *in vitro*. Proc. Soc. Exp. Biol. Med. 33:253-255.

1093. **Steenken, Jr., W., W. H. Oatway, Jr., and S. A. Petroff.** 1934. Biological studies of the tubercle bacillus. III. Dissociation and pathogenicity of the R and S variants of the human tubercle bacillus (H_{37}). J. Exp. Med. 60:515-540.

1094. **Stefansky, W. K.** 1903. Eine lepraähnliche Erkrankung der Haut und der Lymphdrüsen ber Wanderratten. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. I. Abt. Orig. 33:481-487.

1095. **Steward, A. M.** 1973. Tuberculin reaction in cancer patients, "Mantoux release," and lymphosuppressive-stimulatory factors. J. Natl. Cancer Inst. 50:625-632.

1096. **Stewart-Tull, D. E. S.** 1965. Occurrence of dimorphic forms of *Mycobacterium phlei*. Nature (London) 208:603-605.

1097. **Stewart-Tull, D. E. S., J. P. Arbuthnott, and J. H. Freer.** 1975. Some properties of IgG_1 and IgG_2 globulins from normal and adjuvant stimulated guinea-pigs. Immunochemistry 12:941-947.

1098. **Stewart-Tull, D. E. S., T. Shimono, S. Kotani, M. Kato, Y. Ogawa, Y. Yamamura, T. Koga, and C. M. Pearson.** 1975. The adjuvant activity of a non-toxic, water-soluble glycopeptide present in large quantities in the culture filtrate of *Mycobacterium tuberculosis* strain DT. Immunology 29:1-15.

1099. **Stewart-Tull, D. E. S., and R. G. White.** 1964. The occurrence of muramic acid in wax D preparations of mycobacteria. J. Gen. Microbiol. 34:43-49.

1100. **Stewart-Tull, D. E. S., and R. G. White.** 1967. The influence of age of culture on the production of adjuvant-active peptidoglycolipids by saprophytic mycobacteria. Immunology 12:349-359.

1101. **Stewart-Tull, D. E. S., and P. C. Wilkinson.** 1973. The affinity of mycobacterial glycopeptides for guinea-pig $gamma_2$ immunoglobulin and its fragments. Immunology 24:205-216.

1102. **Stewart-Tull, D. E. S., P. C. Wilkinson, and R. G. White.** 1965. The affinity of a mycobacterial glycopeptide for guinea-pig gamma-globulin. Immunology 9:151-160.

1103. **Stodola, F. A., A. Lesuk, and R. J. Anderson.** 1938. The chemistry of the lipids of tubercle bacilli. LIV. The isolation and properties of mycolic acid. J. Biol. Chem. 126:505-513.

1104. **Straus, I., and N. Gamaleia.** 1891. Recherches expérimentales sur la tuberculose; la tuberculose humaine, sa distinction de la tuberculose des oiseaux. Arch. Med. Exp. Anat. Pathol. 3:457-484.

1105. **Subrahmanyam, D., and D. R. Singhvi.** 1966. Phosphatide antigens of mycobacteria. Proc. Soc. Exp. Biol. Med. 120:102-105.

1106. **Suigta, K., T. Tsuda, T. Sakakibara, R. Ushiku, and T. Takahashi.** 1965. Studies on the bacteriophage susceptibility against *Mycobacterium tuberculosis*. Report 1. The difference in lysis range of mycobacteriophage propagated on various host strains. Yokohama Med. Bull. 16:229-235.

1107. **Šula, L.** 1955. The use of an antituberculous vaccine from the *Mycobacterium tuberculosis, typus murinus*-Wells, adapted for deep culture in a synthetic liquid medium. Acta Tuberc. Scand. 31:190-196.

1108. **Šula, L., J. Galliová, and M. Mara.** 1972. A sudden decrease in the lipid content of *Mycobacterium bovis* BCG and *Mycobacterium microti* MP. A preliminary communication. Zentralbl. Bakteriol. Parasitenk. Infektionskrh. Hyg. I. Abt. Orig. A 219:264-265.

1109. **Šula, L., W. B. Redmond, J. F. Coster, I. Baess, J. H. Bates, G. Caroli, E. Mankiewicz, T. Murohashi, and E. Vandra.** 1973. WHO cooperative studies on the phage-typing of mycobacteria: 1. Phage lysis of Czechoslovak and Italian strains of *Mycobacterium tuberculosis*. Bull. W.H.O. 48:57-63.

1110. **Sullivan, P. A.** 1968. Crystallization and properties of L-lactate oxidase from *Mycobacterium smegmatis*. Biochem. J. 110:363-371.

1111. **Sushida, K., and N. Hirano.** 1970. Isolation of bacteriophage of human type tubercle bacilli H37Rv by addition of protamilase (in Japanese). Med. Biol. (Tokyo) 81:221-225.

1112. **Sutherland, I.** 1971. Future policy for BCG vaccination in Britain. Postgrad. Med. J. 47:756-758.

1113. **Sutherland, I.** 1972. Fifty years of BCG. Tubercle 53:150-151.

1114. **Sutton, W. B.** 1957. Mechanism of action and crystallization of lactic oxidative decarboxylase from *Mycobacterium phlei*. J. Biol. Chem. 226:395-405.

1115. **Suzanne, M., and G. Penso.** 1953. Sulla identita' specifica del cosiddetto "ceppo chauvire": *Mycobacterium marianum* n. sp. (Nota preliminare), p. 382-383. VI. Congr. Int. Microbiol., Roma. Riassunti delle communicazione. Vol. II (sez. XII).

1116. **Swift, S., and H. Cohen.** 1962. Granulomas of the skin due to *Mycobacterium balnei* after abrasions from a fish tank. N. Engl. J. Med. 267:1244-1246.

1117. **Tada, T.** 1974. The mode and sites of action of suppressor T cells in the antigen-induced

differentiation of B cells, p. 471-492. *In* D. H. Katz and B. Benacerraf (ed.), Immunological tolerance: mechanisms and potential therapeutic applications. Academic Press Inc., New York.

1118. **Takayama, K.** 1974. Selective action of isoniazid on the synthesis of cell wall mycolates in mycobacteria. Ann. N.Y. Acad. Sci. 235:426-438.
1119. **Takayama, K., and E. L. Armstrong.** 1971. Mannolipid synthesis in a cell-free system of *Mycobacterium smegmatis*. FEBS Lett. 18:67-69.
1120. **Takayama, K., E. L. Armstrong, and H. L. David.** 1974. Restoration of mycolate synthetase activity in *Mycobacterium tuberculosis* exposed to isoniazid. Am. Rev. Resp. Dis. 110:43-48.
1121. **Takayama, K., and D. S. Goldman.** 1969. Pathway for the synthesis of mannophospholipids in *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 176:196-198.
1122. **Takayama, K., and D. S. Goldman.** 1970. Enzymatic synthesis of mannosyl-1-phosphoryl-decarprenol by a cell-free system of *Mycobacterium tuberculosis*. J. Biol. Chem. 245:6251-6257.
1123. **Takayama, K., A. D. Keith, and W. Snipes.** 1975. Effect of isoniazid on the protoplasmic viscosity in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 7:22-24.
1124. **Takayama, K., H. K. Schnoes, and E. J. Semmler.** 1973. Characterization of the alkali-stable mannophospholipids of *Mycobacterium smegmatis*. Biochim. Biophys. Acta 316:212-221.
1125. **Takayama, K., L. Wang, and H. L. David.** 1972. Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 2:29-35.
1126. **Takayama, K., L. Wang, and R. S. Merkal.** 1973. Scanning electron microscopy of the H37Ra strain of *Mycobacterium tuberculosis* exposed to isoniazid. Antimicrob. Agents Chemother. 4:62-65.
1127. **Takemori, S., Y. Nakai, K. Nakazawa, M. Katagiri, and T. Nakamura.** 1973. A lactate oxygenase from *Mycobacterium phlei*. II. Spectral characteristics in aerobic and anaerobic reactions. Arch. Biochem. Biophys. 154:137-146.
1128. **Takemori, S., K. Nakazawa, Y. Nakai, K. Suzuki, and M. Katagiri.** 1968. A lactate oxygenase from *Mycobacterium phlei*. Improved purification and some properties of the enzyme. J. Biol. Chem. 243:313-319.
1129. **Takemori, S., H. Tajima, F. Kawahara, Y. Nakai, and M. Katagiri.** 1974. A lactate oxygenase from *Mycobacterium phlei*. III. Evidence for the subunit structure. Arch. Biochem. Biophys. 160:289-294.
1130. **Takeya, K., and K. Amako.** 1964. The structure of mycobacteriophages. Virology 24:461-466.
1131. **Takeya, K., and K. Hisatsune.** 1963. Mycobacterial cell walls. I. Methods of preparation and treatment with various chemicals. J. Bacteriol. 85:16-23.
1132. **Takeya, K., K. Hisatsune, and Y. Inoue.** 1963. Mycobacterial cell walls. II. Chemical composition of the "basal layer." J. Bacteriol. 85:24-30.
1133. **Takeya, K., M. Koike, R. Mori, Y. Yuda, and T. Toda.** 1959. Light and electron microscope studies of mycobacterium-mycobacteriophage interactions. II. Electron microscope studies. J. Bacteriol. 78:313-319.
1134. **Takeya, K., R. Mori, M. Koike, and T. Toda.** 1958. Paired fibrous structure in mycobacteria. Biochim. Biophys. Acta 30:197-198.
1135. **Takeya, K., R. Mori, T. Tokunaga, M. Koike, and K. Hisatsune.** 1961. Further studies on the paired fibrous-structure of mycobacterial cell wall. J. Biophys. Biochem. Cytol. 9:496-501.
1136. **Takeya, K., and H. Tokiwa.** 1974. Bacteriocin-typing of *Mycobacterium tuberculosis*. Am. Rev. Resp. Dis. 109:304-305.
1137. **Takeya, K., T. Yoshimura, K. Yamaura, and T. Toda.** 1959. Studies on the biologic properties of mycobacteriophage. Am. Rev. Respir. Dis. 80:543-553.
1138. **Tanaka, A.** 1963. Fractionation of wax D, a peptidoglycolipid of *Mycobacterium tuberculosis*. Biochim. Biophys. Acta 70:483-485.
1139. **Tanaka, A., T. Nagasaki, S. Fukui, and S. Murakami.** 1969. Studies on the formation of vitamins and their functions in hydrocarbon fermentation. VII. Electronmicroscopical studies on hydrocarbon-grown and glucose-grown cells of *Mycobacterium smegmatis*. J. Ferment. Technol. 47:739-743.
1140. **Tárnok, I., E. Röhrscheidt, und R. Bönicke.** 1967. Basenzusammensetzung der Desoxyribonucleinsäure (DNS) von Mykobakterien und verwandten Mikroorganismen. Rass. Patol. Apparat. Respirator. 17:375-388.
1141. **Tárnok, I., and Z. Tárnok.** 1970. Carotenes and xanthophylls in mycobacteria. I. Technical procedures; thin-layer chromatographic patterns of mycobacterial pigments. Tubercle 51:305-312.
1142. **Tárnok, I., and Z. Tárnok.** 1971. Carotenes and xanthophylls in mycobacteria. II. Lycopenes, α- and β-carotene and xanthophyll in mycobacterial pigments. Tubercle 52:127-135.
1143. **Taub, R. N., and R. K. Gershon.** 1972. The effect of localized injection of adjuvant material on the draining lymph node. III. Thymus dependence J. Immunol. 108:377-386.
1144. **Taub, R. N., R. Krantz, and D. W. Dresser.** 1970. The effect of localized injection of adjuvant material on the draining lymph node. I. Histology. Immunology 18:171-186.
1145. **Taussig, M. J.** 1973. Antigenic competition. Curr. Top. Microbiol. Immunol. 60:125-174.
1146. **Taussig, M. J.** 1974. The effect of Freund's

complete adjuvant on the specificity of an immune response and its relationship to antigenic competition. Cell. Immunol. 11:484–487.

1147. **Thacore, H., and H. P. Willett.** 1966. The formation of spheroplasts of *Mycobacterium tuberculosis* in tissue culture cells. Am. Rev. Respir. Dis. 93:786–796.

1148. **Thestrup-Pedersen, K.** 1974. Temporary suppression of lymphocyte transformation after tuberculin skin testing. Immunology 27:965–971.

1149. **Thestrup-Pedersen, K.** 1975. Suppression of tuberculin skin reactivity by prior tuberculin skin testing. Immunology 28:343–348.

1150. **Thewaini Ali, A. J., and C. L. Oakley.** 1967. Auto-antibodies in experimental chronic infective disease. J. Pathol. Bacteriol. 93:413–433.

1151. **Thin, G.** 1883. On the bacillus of leprosy. Br. Med. J. 1:1178–1179.

1151a. **Thoen, C. O., A. G. Karlson, and R. D. Ellefson.** 1972. Serum lipid-lipoprotein profiles in rabbits infected with *Mycobacterium bovis, Listeria monocytogenes, Pasteurella pseudotuberculosis* and with *M. avium*. Mayo Clin. Proc. 47:258–269.

1151b. **Thoen, C. O., A. G. Karlson, and R. D. Ellefson.** 1972. Mechanisms of changes in serum lipid-lipoprotein profiles in infectious disease. *Mycobacterium bovis* infection in rabbits. Mayo Clin. Proc. 47:270–272.

1152. **Thomas, L.** 1959. Reactions to homologous tissue antigens in relation to hypersensitivity, p. 529–532. *In* H. S. Lawrence (ed.), Cellular and humoral aspects of the hypersensitive states. Hoeber-Harper, New York.

1153. **Thompson, J. S., C. D. Severson, N. A. Stearns, and J. E. Kasik.** 1972. Immunological distinction of mycobacterial beta-lactamases. Infect. Immun. 5:542–546.

1154. **Toida, I.** 1973. Effects of cord factor on pyrazinamide deamidase. Am. Rev. Respir. Dis. 108:694–697.

1155. **Toida, I.** 1974. Effects of cord factor on microsomal enzymes. Am. Rev. Respir. Dis. 110:641–646.

1156. **Tokunaga, T., Y. Maruyama, and T. Murohashi.** 1963. Further studies on the phage typing of rapidly growing mycobacteria. Jpn. J. Bacteriol. 20:554–559.

1157. **Tokunaga, T., Y. Maruyama, and T. Murohashi.** 1968. Classification of subtypes of human tubercle bacilli by phage susceptibility. Am. Rev. Respir. Dis. 97:469–471.

1158. **Tokunaga, T., and M. I. Sellers.** 1970. Transfection of amino acid-sensitized mycobacteria, p. 152–165. *In* S. E. Juhasz and G. Plummer (ed.), Host-virus relationships in *Mycobacterium, Nocardia* and *Actinomyces*. Charles C Thomas, Publisher, Springfield, Ill.

1159. **Toussaint, A. J., E. H. Fife, R. C. Parlett, L. F. Affronti, G. L. Wright, M. Reich, and W. C. Morse.** 1969. A soluble antigen fluorescent antibody test for the serodiagnosis of *Mycobacterium tuberculosis* infection. Am. J. Clin. Pathol. 52:708–713.

1160. **Tsang, A. Y., and E. R. Farber.** 1973. The primary isolation of *Mycobacterium ulcerans*. Am. J. Clin. Pathol. 59:688–692.

1161. **Tsuji, S., K. Ito, and S. Oshima.** 1957. The role of humoral factors in native and acquired resistance to tuberculosis. Am. Rev. Tuberc. Pulmon. Dis. 76:90–102.

1162. **Tsukamura, M.** 1965. A group of mycobacteria from soil sources resembling nonphotochromogens (group 3). A description of *Mycobacterium nonchromogenicum*. Med. Biol. (Tokyo) 71:110–113.

1163. **Tsukamura, M.** 1965. A thermoresistant, mycobacterial strain, probably a new species (in Japanese). Med. Biol. (Tokyo) 71:52–54.

1164. **Tsukamura, M.** 1966. Adansonian classification of mycobacteria. J. Gen. Microbiol. 45:253–273.

1165. **Tsukamura, M.** 1967. Identification of mycobacteria. Tubercle 48:311–338.

1166. **Tsukamura, M.** 1967. *Mycobacterium chitae:* a new species. Jpn. J. Microbiol. 11:43–47.

1167. **Tsukamura, M.** 1967. Two types of slowly growing, nonphotochromogenic mycobacteria obtained from soil by the mouse passage method: *Mycobacterium terrae* and *Mycobacterium novum*. Jpn. J. Microbiol. 11:163–172.

1168. **Tsukamura, M.** 1970. Variability of the pigmentation of mycobacteria by the composition of medium. Pneumonologie 142:93–101.

1168a. **Tsukamura, M.** 1976. Numerical classification of slowly growing mycobacteria. Int. J. Syst. Bacteriol. 26:409–420.

1169. **Tsukamura, M., and S. Mizuno.** 1968. "Hypothetical mean organisms" of mycobacteria. A study of classification of mycobacteria. Jpn. J. Microbiol. 12:371–384.

1170. **Tsukamura, M., and S. Mizuno.** 1971. *Mycobacterium obuense,* a rapidly growing scotochromogenic mycobacterium capable of forming a black product from *p*-aminosalicylate and salicylate. J. Gen. Microbiol. 68:129–134.

1171. **Tsukamura, M., S. Mizuno, N. F. F. Gane, A. Mills, and L. King.** 1971. *Mycobacterium rhodesiae* sp. nov. Jpn. J. Microbiol. 15:407–416.

1172. **Tsukamura, M., S. Mizuno, and S. Tsukamura.** 1968. Classification of rapidly growing mycobacteria. Jpn. J. Microbiol. 12:151–166.

1173. **Tsukamura, M., E. Nakamura, I. Kurita, and T. Nakamura.** 1973. Isolation of *Mycobacterium chelonei* subspecies *chelonei* (*Mycobacterium borstelense*) from pulmonary lesions of 9 patients. Am. Rev. Respir. Dis. 108:683–685.

1173a. **Tsukamura, M., H. Shimoide, and W. B. Shaefer.** 1975. A possible new pathogen of group III mycobacteria. J. Gen. Microbiol.

88:377-380.
1174. Tsukamura, M., H. Toyama, and S. Mizuno. 1965. *Mycobacterium parafortuitum*, a new species. Med. Biol. (Tokyo) 71:232-235.
1175. Tsukamura, M., and S. Tsukamura. 1966. *Mycobacterium aurum*, a new species. Med. Biol. 72:270-273.
1176. Tsukamura, M., and S. Tsukamura. 1967. A practical system for identification of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii* and *Mycobacterium fortuitum*. Scand. J. Resp. Dis. 48:58-70.
1177. Tsukamura, S. 1964. Biological significance of pigments of mycobacteria. I. Artificial induction of pigmentless mutants from photochromogens and scotochromogens by ultraviolet irradiation. II. Correlation of pigment formation with the virulence. Jpn. J. Tuberc. 12:1-6.
1178. Tsukamura, S. 1964. Biological significance of pigments of mycobacteria. III. Resistance to ultraviolet irradiation of various unclassified mycobacteria. Jpn. J. Tuberc. 12:7-9.
1179. Tung, K.-K., and C. E. Ballou. 1973. Biosynthesis of a mycobacterial lipopolysaccharide. Properties of the polysaccharide:acyl coenzyme A acyltransferase reaction. J. Biol. Chem. 248:7126-7133.
1180. Turcotte, R. 1969. The variations in the antigenic composition of *Mycobacterium tuberculosis* during the growth cycle as measured by passive hemagglutination and precipitation reactions. Can. J. Microbiol. 15:681-688.
1181. Turcotte, R., and R. P. Boulanger. 1971. Comparison between the antigenic components extracted from virulent and avirulent strains of mycobacteria. Can. J. Microbiol. 17:95-100.
1182. Turian, G. 1950. Recherches sur la biosynthèse des caroténoides chez un Bacille paratuberculeux. III. Inhibition de la pigmentation par la diphenylamine. Helv. Chim. Acta 33:1988-1993.
1183. Turian, G., and F. Haxo. 1952. Further use of diphenylamine for the study of carotenoid biosynthesis in *Mycobacterium phlei*. J. Bacteriol. 63:690-691.
1184. Turk, J. L., and A. D. M. Bryceson. 1971. Immunological phenomena in leprosy and related diseases. Adv. Immunol. 13:209-266.
1185. Turk, J. L., L. Polak and D. Parker. 1976. Control mechanisms in delayed-type hypersensitivity. Br. Med. Bull. 32:165-170.
1186. Turner, L. 1970. Atypical mycobacterial infections in ophthalmology. Trans. Am. Ophthal. Soc. 68:667-729.
1187. Turner, L., and I. Stinson. 1965. *Mycobacterium fortuitum* as a cause of corneal ulcer. Am. J. Ophthalmol. 60:329-331.
1188. Twort, F. W. 1911. A method for isolating and growing the lepra bacillus of man and the bacillus of Johne's disease in cattle. (Preliminary note). Vet. J. 67:118-120.
1189. Twort, F. W., and G. L. Y. Ingram. 1913. A monograph on Johne's disease. Bailliere, Tindall and Cox, London.
1190. Uhr, J. W. 1966. Delayed hypersensitivity. Physiol. Rev. 46:359-419.
1191. Unanue, E. R. 1970. Thymus dependency of the immune response to hemocyanin: an evaluation of the role of macrophages in thymectomized mice. J. Immunol. 105:1339-1343.
1192. Unanue, E. R., and J. C. Cerottini. 1970. The immunogenicity of antigen bound to the plasma membrane of macrophages. J. Exp. Med. 131:711-725.
1193. Vacheron, M.-J., M. Guinand, G. Michel, and J.-M. Ghuysen. 1972. Structural investigations on cell walls of *Nocardia* sp. The wall lipid and peptidoglycan moieties of *Nocardia kirovani*. Eur. J. Biochem. 29:156-166.
1194. Vagelos, P. R. 1959. Caproyl coenzyme A dependent malonyl coenzyme A - bicarbonate exchange reaction. J. Am. Chem. Soc. 81:4119.
1195. Valdivia, J. A. 1973. *Mycobacterium habana*: clinical and epidemiological significance. Am. Soc. Belge Med. Trop. 53:263-266.
1196. Valdivia Alvarez, J., R. S. Mendez, and M. E. Font. 1971. *Mycobacterium habana*: probable nueva especie dentro de la micobacterias no clasificadas. Bol. Hig. Epidemiol. 9:65-73.
1197. Vance, D. E., T. W. Esders, and K. Bloch. 1973. On the role of a palmityl thioesterase in fatty acid elongation. J. Biol. Chem. 248:2310-2316.
1198. Vance, D. E., O. Mitsuhashi, and K. Bloch. 1973. Purification and properties of the fatty acid synthetase from *Mycobacterium phlei*. J. Biol. Chem. 248:2303-2309.
1199. Vandepitte, J., J. Desmyter, and F. Gatti. 1969. Mycobacteria, skins, and needles. Lancet 2:691.
1200. Vandra, E. 1970. Untersuchungen über die Phagenadsorption bei lysogenen Mycobakterienstämmen. Pneumonologie 142:158-164.
1201. Vandra, E., and T. Fodor. 1971. Phage typing of *Mycobacterium tuberculosis* strains isolated in Hungary. Acta Microbiol. Acad. Sci. Hung. 18:155-158.
1202. van Furth, R. and Z. A. Cohn. 1968. The origin and kinetics of mononuclear phagocytes. J. Exp. Med. 128:415-433.
1203. Vestal, A. L. 1975. Procedures for the isolation and identification of mycobacteria. U.S. Department of Health, Education and Welfare publication no. (CDC) 75-8230. U.S. Government Printing Office, Washington, D.C.
1204. Vilkas, E., C. Amar, J. Markovits, J. F. G. Vliegenthart, and J. P. Kamerling. 1973. Occurrence of a galactofuranose disaccharide in immunoadjuvant fractions of *Mycobacterium tuberculosis* (cell walls and wax

D). Biochim. Biophys. Acta 297:423–435.
1205. Vilkas, E., C. Gros, and J.-C. Massot. 1968. Sur la structure chimique d'un mycoside C isolé de *Mycobacterium scrofulaceum*. C. R. Acad. Sci. C (Paris) 266:837–840.
1206. Vilkas, E., and E. Lederer. 1968. *N*-methylation de peptides par la methode de Hakomori. Structure du mycoside C_{b1}. Tetrah. Lett. 26:3089–3092.
1207. Villé, C., and M. Gastambide-Odier. 1970. Le 3-*O*-méthyl-L-rhamnose, sucre isolé du mycoside G de *Mycobacterium marinum*. Carbohydr. Res. 12:97–107.
1208. Virgilio, L. A., and W. W. Johnston. 1973. Fatal pulmonary infection with *Mycobacterium fortuitum*. N. C. Med. J. 34:796–798.
1209. Voiland, A., M. Bruneteau, and G. Michel. 1971. Etude du mycoside C_2 de *Mycobacterium avium*. Détermination de la structure. Eur. J. Biochem. 21:285–291.
1210. Volini, F., R. Colton, and W. Lester. 1965. Disseminated infection caused by Battey type mycobacteria. Am. J. Clin. Pathol. 43:39–46.
1211. Volpe, J. J., and P. R. Vagelos. 1973. Saturated fatty acid biosynthesis and its regulation. Annu. Rev. Biochem. 42:21–60.
1211a. Wager, O. 1975. Immunopathogenic mechanisms in rheumatoid diseases with special reference to IgM rheumatoid factor. Scand. J. Rheumatol. 12(Suppl.):67–76.
1211b. Wagstaff, M., A. Broughton, and F. R. Jones. 1976. The reaction kinetics of gastric intrinsic factor and IgG intrinsic factor blocking antibody: a soluble system. Immunochemistry 13:437–441.
1212. Wahl, S. M., G. M. Iverson, and J. J. Oppenheim. 1974. Induction of guinea pig B-cell lymphokine synthesis by mitogenic and nonmitogenic signals to Fc, Ig, and C3 receptors. J. Exp. Med. 140:1631–1645.
1213. Wakil, S. J. 1958. A malonic acid derivative as an intermediate in fatty acid synthesis. J. Am. Chem. Soc. 80:6465.
1213a. Waksman, B. H., and Y. Namba. 1976. On soluble mediators of immunologic regulation. Cell. Immunol. 21:161–176.
1214. Waksman, B. H., C. H. Pearson, and J. T. Sharp. 1960. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. J. Immunol. 85:403–417.
1215. Walker, H. H., M. F. Shinn, M. Higaki, and J. Ogata. 1962. Some characteristics of "swimming pool" disease in Hawaii. Hawaii Med. J. 21:403–409.
1216. Walker, R. W., H. Barakat, and J. G. C. Hung. 1970. The positional distribution of fatty acids in the phospholipids and triglycerides of *Mycobacterium smegmatis* and *M. bovis* BCG. Lipids 5:684–691.
1217. Walker, R. W., J.-C. Prome, and C. S. Lacave. 1973. Biosynthesis of mycolic acids. Formation of a C_{32}/β-keto ester from palmitic acid on a cell free system of *Corynebacterium diphtheriae*. Biochim. Biophys. Acta 326:52–62.
1218. Wallen, W. C., J. H. Dean, and D. A. Lucas. 1973. Interferon and the cellular immune response: separation of interferon-producing cells from DNA-synthesizing cells. Cell. Immunol. 6:110–122.
1219. Wang, L., T. Kusaka, and D. S. Goldman. 1970. Elongation of fatty acids in *Mycobacterium tuberculosis*. J. Bacteriol. 101:781–785.
1220. Wang, L., and K. Takayama. 1972. Relationship between the uptake of isoniazid and its action on in vivo mycolic acid synthesis in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 2:438–441.
1221. Ward, D. E. 1970. Buruli ulcer. Br. Med. J. 3:346.
1221a. Warner, N. L. 1974. Membrane immunoglobulins and antigen receptors on B and T lymphocytes. Adv. Immunol. 19:67–216.
1222. Wassermann, H. E. 1973. Avian tuberculosis endophthalmitis. Arch. Ophthalmol. 89: 321–323.
1223. Watanakunakorn, C., and A. Trott. 1973. Vertebral osteomyelitis due to *Mycobacterium kansasii*. Am. Rev. Respir. Dis. 107:846–850.
1224. Watkinson, R. J., H. Hussey, and J. Baddiley. 1971. Shared lipid phosphate carrier in the biosynthesis of teichoic acid and peptidoglycan. Nature (London) New Biol. 229:57–59.
1225. Wayne, L. G. 1966. Classification and identification of mycobacteria. III. Species within group III. Am. Rev. Respir. Dis. 93:919–928.
1226. Wayne, L. G. 1970. On the identity of *Mycobacterium gordonae* Bojalil and the so-called tap water scotochromogens. Int. J. Syst. Bacteriol. 20:149–153.
1227. Wayne, L. G. 1971. Phenol-soluble antigens from *Mycobacterium kansasii, Mycobacterium gastri* and *Mycobacterium marinum*. Infect. Immun. 3:36–40.
1228. Wayne, L. G. 1975. Proposal to reject the specific epithet *marianum* in the name *Mycobacterium marianum* Penso 1953 and to conserve the specific epithet *scrofulaceum* in the name *Mycobacterium scrofulaceum* Prissick and Masson 1956. Request for an opinion. Int. J. Syst. Bacteriol. 25:230–231.
1229. Wayne, L. G., and G. A. Diaz. 1967. Autolysis and secondary growth of *Mycobacterium tuberculosis* in submerged culture. J. Bacteriol. 93:1374–1381.
1230. Wayne, L. G., and G. A. Diaz. 1976. Immunoprecipitation studies of mycobacterial catalase. Int. J. Syst. Bacteriol. 26:38–44.
1231. Wayne, L. G., T. M. Dietz, C. Gernez-Rieux, P. A. Jenkins, W. Käppler, G. P. Kubica, J. B. G. Kwapinski, G. Meissner, S. R. Pattyn, E. H. Runyon, K. H. Schröder, V. A. Silcox, A. Tacquet, M. Tsukamura, and E.

Wolinsky. 1971. A co-operative numerical analysis of scotochromogenic slowly growing mycobacteria. J. Gen. Microbiol. 66:255-271.

1232. Wayne, L. G., J. R. Doubek, and G. A. Diaz. 1967. Classification and identification of mycobacteria. IV. Some important scotochromogens. Am. Rev. Respir. Dis. 96:88-95.

1232a. Wayne, L. G., H. C. Engbaek, H. W. B. Engel, S. Froman, W. Gross, J. Hawkins, W. Käppler, A. G. Karlson, H. H. Kleeberg, I. Krasnow, G. P. Kubica, C. McDurmont, E. E. Nel, S. R. Pattyn, K. H. Schröder, S. Showalter, I. Tarnok, M. Tsukamura, B. Vergman and E. Wolinsky. 1974. Highly reproducible techniques for use in systematic bacteriology in the genus *Mycobacterium*: test for pigment, urease, resistance to sodium chloride, hydrolysis of Tween 80 and β-galactosidase. Int. J. Syst. Bacteriol. 24:412-419.

1232b. Wayne, L. G., H. W. B. Engel, C. Grassi, W. Gross, J. Hawkins, P. A. Jenkins, W. Käppler, H. H. Kleeberg, I. Krasnow, E. E. Nel, S. R. Pattyn, P. A. Richards, S. Showalter, M. Slosarek, I. Szabo, I. Tarnok, M. Tsukamura, B. Vergmann, and E. Wolinsky. 1976. Highly reproducible techniques for use in systematic bacteriology in the genus *Mycobacterium*: tests for niacin and catalase and for resistance to isoniazid, thiophene 2-carboxylic acid hydrazide, hydroxylamine, and *p*-nitrobenzoate. Int. J. Syst. Bacteriol. 26:311-318.

1233. Wayne, L. G., and W. M. Gross. 1968. Base composition of deoxyribonucleic acid isolated from mycobacteria. J. Bacteriol. 96:1915-1919.

1234. Wayne, L. G., and E. F. Lessel. 1969. On the synonymy of *Mycobacterium marianum* Penso 1953 and *Mycobacterium scrofulaceum* Prissick and Masson 1956 and the resolution of a nomenclatural problem. Int. J. Syst. Bacteriol. 19:257-261.

1235. Wayne, L. G., E. H. Runyon, and G. P. Kubica. 1969. Mycobacteria: a guide to nomenclatural usage. Am. Rev. Respir. Dis. 100:732-734.

1236. Weedon, B. C. L. 1971. Stereochemistry, p. 267-323. *In* O. Isler (ed.), Carotenoids. Birkhäuser Verlag, Basel; Halsted Press, John Wiley & Sons, New York.

1237. Wehrli, W., and M. Staehelin. 1971. Actions of the rifamycins. Bacteriol. Rev. 35:290-309.

1238. Weiner, I. M., T. Higuchi, L. Rothfield, M. Saltmarsh-Andrew, M. J. Osborn, and B. L. Horecker. 1965. Biosynthesis of bacterial lipopolysaccharide. V. Lipid-linked intermediates in the biosynthesis of the O-antigenic groups of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. 54:228-235.

1239. Weir, M. P., W. H. R. Langridge III, and R. W. Walker. 1972. Relationships between oleic acid uptake and lipid metabolism in *Mycobacterium smegmatis*. Am. Rev. Respir. Dis. 106:450-457.

1240. Weiss, D. W., and D. J. Yashphe. 1973. Nonspecific stimulation of antimicrobial and antitumor resistance and of immunological responsiveness by the MER fraction of tubercle bacilli, p. 163-223. *In* A. Zukerman and D. W. Weiss (ed.), Dynamic aspects of host-parasite relationships, vol. 1. Academic Press Inc., New York.

1241. Weissfieler, J., V. Karassova, and J. Holland. 1964. Atypical mycobacteria in monkeys. Acta Microbiol. Acad. Sci. Hung. 11:403-407.

1242. Weiszfieler, J. G. 1973. Problems of atypical mycobacteria, p. 13-22. *In* J. G. Weiszfeiler (ed.), Atypical mycobacteria. Akadémiae Kiadó, Budapest.

1243. Weiszfieler, J., I. Jokay, E. Karczag, K. Almassy, and P. Somos. 1968. Taxonomic studies on mycobacteria on the basis of their antigenic structure. Acta Microbiol. Acad. Sci. Hung. 15:69-76.

1244. Weiszfeiler, J. G., V. Karasseva, and E. Karczag. 1970. Studies on *Mycobacterium simiae* and some other atypical mycobacteria isolated from monkeys, p. 31-39. *In* J. G. Weiszfeiler (ed.), Proc. Microbiol. Res. Group Hung. Acad. Sci., Vol. III. Akadémiai Kiadó, Budapest.

1245. Weiszfeiler, J. G., J. Karasseva, and E. Karczag. 1971. A new Mycobacterium species: *Mycobacterium asiaticum* n. sp. Acta Microbiol. Acad. Sci. Hung. 18:247-252.

1245a. Weiszfeiler, J. G., and E. Karczag. 1976. Synonymy of *Mycobacterium simiae* Karasseva et al. 1965 and *Mycobacterium habana* Valdiva et al. 1971. Int. J. Syst. Bacteriol. 26:474-477.

1246. Wells, A. Q. 1937. Tuberculosis in wild voles. Lancet 232:1221.

1247. Wells, A. Q. 1957. The vole bacillus vaccine. Adv. Tuberc. Res. 8:61-78.

1248. Wells, A. Q., E. Agius, and N. Smith. 1955. *Mycobacterium fortuitum*. Am. Res. Tuberc. Pulmon. Dis. 72:53-63.

1249. Wells, A. Q., and J. A. H. Wylie. 1954. Vaccination against tuberculosis with the vole bacillus. Br. Med. Bull. 10:96-99.

1250. White, H. B. III, O. Mitsuhashi, and K. Bloch. 1971. Pyridine nucleotide requirements of fatty acid synthetases. J. Biol. Chem. 246:4751-4754.

1251. White, R. G., A. H. Coons, and J. M. Connolly. 1955. Studies on antibody production. IV. The role of a wax fraction of *Mycobacterium tuberculosis* in adjuvant emulsions on the production of antibody to egg albumin. J. Exp. Med. 102:83-104.

1252. White, R. G., and A. H. E. Marshall. 1958. The role of various chemical fractions of *M. tuberculosis* and other mycobacteria in the production of allergic encephalomyelitis. Immunology 1:111-122.

1253. White, R. J., G. C. Lancini, and L. G. Silves-

tri. 1971. Mechanism of action of rifampin on *Mycobacterium smegmatis*. J. Bacteriol. 108:737-741.

1254. **Wiant, J. R.** 1971. Mycobacterial diseases. Ariz. Med. 28:96-100.

1255. **Wieland, T.** 1968. Poisonous principles of mushrooms of the genus *Amanita*. Four-carbon amines acting on the central nervous system and cell-destroying cyclic peptides are produced. Science 159:946-952.

1256. **Wietzerbin, J., B. C. Das, J.-F. Petit, E. Lederer, M. Leyh-Bouille, and J. M. Ghuysen.** 1974. Occurrence of D-alanyl-(D)-*meso*-diaminopimelic acid and *meso*-diaminopimelyl-*meso*-diaminopimelic acid interpeptide linkages in the peptidoglycan of *Mycobacteria*. Biochemistry 13:3471-3475.

1257. **Wietzerbin-Falszpan, J., B. C. Das, I. Azuma, A. Adam. J.-F. Petit, and E. Lederer.** 1970. Isolation and mass spectrometric identification of the peptide subunits of mycobacterial cell walls. Biochem. Biophys. Res. Commun. 40:57-63.

1258. **Wietzerbin-Falszpan, J., B. C. Das, C. Gros, J.-F. Petit, and E. Lederer.** 1973. The amino acids of the cell wall of *Mycobacterium tuberculosis* var. *bovis*, strain BCG. Presence of a poly (L-glutamic acid). Eur. J. Biochem. 32:525-532.

1259. **Wigle, W. D., M. J. Ashley, E. M. Killough, and M. Cosens.** 1972. Bovine tuberculosis in humans in Ontario. The epidemiological features of 31 cases occurring between 1964 and 1970. Am. Rev. Respir. Dis. 106:528-534.

1260. **Wilkinson, P. C.** 1966. Adjuvant granuloma and its effect on the immune response in the guinea pig. Pathol. Eur. 1:204-216.

1261. **Willett, H. P.** 1960. The production of lysine from diaminopimelic acid by cell-free extracts of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 81:653-659.

1262. **Willett, H. P., and H. Thacore.** 1966. The induction by lysozyme of an L-type growth in *Mycobacterium tuberculosis*. Can. J. Microbiol. 12:11-16.

1263. **Willett, H. P., and H. Thacore.** 1967. Formation of spheroplasts of *Mycobacterium tuberculosis* by lysozyme in combination with certain enzymes of rabbit peritoneal monocytes. Can. J. Microbiol. 13:481-488.

1264. **Williams, C. S., and D. C. Riordan.** 1973. *Mycobacterium marinum* (atypical acid-fast bacillus) infections of the hand. A report of six cases. J. Bone Jt. Surg. 55-A:1042-1050.

1264a. **Williams, R. M., R. N. Germain, and B. Benacerraf.** 1975. Specific and nonspecific antitumor immunity. I. Description of an in vitro assay based on inhibition of DNA synthesis in tumor cells. J. Natl. Cancer Inst. 54:697-708.

1265. **Wilson, G. S.** 1925. The serological classification of the tubercle bacilli by agglutination and adsorption of agglutinins. J. Pathol. Bacteriol. 28:69-97.

1266. **Wilson, G. S., and A. A. Miles.** 1955. Topley and Wilson's principles of bacteriology and immunity, vol. 1, 4th ed., p. 519. Williams & Wilkins, Baltimore.

1267. **Wilson, G. S., and A. A. Miles.** 1964. Topley and Wilson's principles of bacteriology and immunity, vol. 1, 5th ed. Williams & Wilkins Co., Baltimore.

1268. **Winblad, B., and M. Duchek.** 1973. Comparison between microscopical methods and cultivation for demonstration of tubercle bacilli in experimental tuberculous infection. Acta Pathol. Microbiol. Scand. A 81:824-830.

1269. **Winblad, S., and C. Erickson.** 1973. Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. Acta Pathol. Microbiol. Scand. 81B:150-156.

1270. **Winder, F. G., and P. B. Collins.** 1970. Inhibition by isoniazid of synthesis of mycolic acids in *Mycobacterium tuberculosis*. J. Gen. Microbiol. 63:41-48.

1271. **Winder, F. G., J. J. Tighe, and P. J. Brennan.** 1972. Turnover of acylglucose, acyltrahalose and free trehalose during growth of *Mycobacterium smegmatis* on glucose. J. Gen. Microbiol. 73:539-546.

1272. **Winslow, C.-E. A., J. Broadhurst, R. E. Buchanan, C. Krumwiede, Jr., L. A. Rogers, and G. H. Smith.** 1920. The families and genera of the bacteria. Final report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types. J. Bacteriol. 5:191-229.

1273. **Winter, F. E., and E. H. Runyon.** 1965. Prepatellan bursitis caused by *Mycobacterium marinum* (*balnei*). Case report, classification, and review of the literature. J. Bone Jt. Surg. 47-A:375-399.

1274. **Witts, L. J.** 1958. Arthur Quinton Wells, 22nd June 1896-9th October 1956. J. Pathol. Bacteriol. 75:495-501.

1274a. **Wolfe, S. A., D. E. Tracey, and C. S. Henney.** 1976. Induction of "natural killer" cells by BCG. Nature (London) 262:584-586.

1275. **Wolinsky, E.** 1963. The role of scotochromogenic mycobacteria in human disease. Ann. N.Y. Acad. Sci. 106:67-71.

1276. **Wolinsky, E.** 1974. Nontuberculous mycobacterial infections of man. Med. Clin. N. Am. 58:639-648.

1277. **Wolinsky, E., F. Gomez, and F. Zimpfer.** 1972. Sporotrichoid *Mycobacterium marinum* infection treated with rifampin-ethambutol. Am. Rev. Respir. Dis. 105:964-967.

1278. **Wolinsky, E., V. N. Kapur, and T. K. Rynearson.** 1967. Avian tubercle bacillus infection in a patient with silicosis. Am. Rev. Respir. Dis. 96:1229-1232.

1279. **Wolinsky, E., and W. Schaefer.** 1973. Proposed numbering scheme for mycobacterial serotypes by agglutination. Int. J. Syst. Bacteriol. 23:182-183.

1280. Wolstenholme, G. E. W., and J. Knight (ed.). 1973. Immunopotentiation. Ciba Foundation Symposium 18 (new series). Associated Scientific Publishers, Amsterdam.
1281. Wood, L. E., V. B. Buhler, and A. Pollak. 1956. Human infection with the "yellow" acid-fast bacillus. A report of fifteen additional cases. Am. Rev. Tuberc. Pulmon. Dis. 73:917-929.
1282. Worcel, A., D. S. Goldman, and I. B. Sachs. 1968. Properties and fine structure of the ribosomes from *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U.S.A. 61:122-129.
1283. World Health Organization Expert Committee on Biological Standards. 1966. Requirements for dried BCG vaccine (requirements for biological substances number 11) 18th report. WHO Tech. Rep. Ser. 329:25-51.
1284. Wright, A. 1971. Mechanism of conversion of the *Salmonella* O antigen by bacteriophage ϵ^{34}. J. Bacteriol. 105:927-936.
1285. Wright, A., M. Dankert, P. Fennessey, and P. W. Robbins. 1967. Characterization of a polyisoprenoid compound functional in O-antigen biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 57:1798-1803.
1286. Wright, G. L., Jr., L. F. Affronti, and M. Reich. 1972. Characterization and comparison of mycobacterial antigens by two-dimensional polyacrylamide gel electrophoresis. Infect. Immun. 5:482-490.
1287. Wright, G. L., Jr., and D. B. Roberts. 1974. Two-dimensional immunoelectrophoresis of mycobacterial antigens. Comparison with a reference system. Am. Rev. Respir. Dis. 109:306-310.
1288. Wunsch, S. E., G. L. Boyle, I. A. Leopold, and M. L. Littman. 1969. *Mycobacterium fortuitum* infection of corneal graft. Arch. Ophthalmol. 82:602-607.
1289. Yamaguchi, B. T., Jr., and H. Braunstein. 1965. Fluorescent stain for tubercle bacilli in histologic sections. II. Diagnostic efficiency in granulomatous lesions of the liver. Am. J. Clin. Pathol. 43:184-187.
1290. Yamaguchi, T. 1965. Comparison of the cell wall composition of morphologically distinct actinomycetes. J. Bacteriol. 89:444-453.
1291. Yamamura, Y., Y. Ogawa, H. Maeda, and Y. Yamamura. 1974. Prevention of tuberculous cavity formation by desensitization with tuberculin-active peptide. Am. Rev. Respir. Dis. 109:594-601.
1292. Yarkoni, E., A. Bekierkunst, J. Asselineau, R. Toubiana, and E. Lederer. 1973. Suppression of growth of Ehrlich ascites tumor cells in mice pretreated with synthetic analogs of trehalose-6,6-dimycolate (cord factor). J. Natl. Canc. Inst. 51:717-720.
1293. Yeager, H., Jr., and J. W. Raleigh. 1973. Pulmonary disease due to *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. 108:547-552.
1294. Yegian, D., and R. J. Vanderlinde. 1947. The nature of acid-fastness. J. Bacteriol. 54:777-783.
1295. Yoder, W. D., and W. B. Schaefer. 1971. Comparison of the seroagglutination test with the pathogenicity test in the chicken for the identification of *Mycobacterium avium* and *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. 103:173-178.
1296. Yoneda, M., and Y. Fukui. 1965. Isolation, purification, and characterization of extracellular antigens of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 92(part 2):9-18.
1297. Yoneda, M., and M. Kondo. 1959. Studies on poly-β-hydroxybutyrate in bacterial spores. 1. Existence of poly-β-hydroxybutyrate in mature spores of a strain of *Bacillus cereus* and its relation to the acid-fast stainability. Biken J. 2:247-258.
1298. Youatt, J. 1969. A review of the action of isoniazid. Am. Rev. Respir. Dis. 99:729-749.
1299. Youatt, J., and G. M. Renwick. 1972. Pigments produced by *Mycobacterium tuberculosis* in the presence of isoniazid. Austr. J. Chem. 25:1585-1593.
1300. Youmans, A. S., and G. P. Youmans. 1965. Immunogenic activity of a ribosomal fraction obtained from *Mycobacterium tuberculosis*. J. Bacteriol. 89:1291-1298.
1301. Youmans, A. S., and G. P. Youmans. 1966. Effect of trypsin and ribonuclease on the immunogenic activity of ribosomes and ribonucleic acid isolated from *Mycobacterium tuberculosis*. J. Bacteriol. 91:2146-2154.
1302. Youmans, A. S., and G. P. Youmans. 1973. The relationship between sedimentation value and immunogenic activity of mycobacterial ribonucleic acid. J. Immunol. 110:581-586.
1303. Youmans, G. P. 1963. The pathogenic "atypical" mycobacteria. Annu. Rev. Microbiol. 17:473-494.
1304. Youmans, G. P. 1975. Relation between delayed hypersensitivity and immunity in tuberculosis. Am. Rev. Respir. Dis. 111:109-118.
1305. Youngner, J. S., and S. B. Salvin. 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J. Immunol. 111:1914-1922.
1306. Zalokar, M. 1955. Biosynthesis of carotenoids in *Neurospora* action spectrum of photoactivation. Arch. Biochem. Biophys. 56:318-325.
1307. Zalokar, M. 1957. Variations in the production of carotenoids in *Neurospora*. Arch. Biochem. Biophys. 70:561-567.
1308. Zbar, B., H. J. Rapp, and E. E. Ribi. 1972. Tumor suppression by cell walls of *Mycobacterium bovis* attached to oil droplets. J. Natl. Cancer Inst. 48:831-835.
1309. Zbar, B., and T. Tanaka. 1971. Immunotherapy of cancer: regression of tumors after intralesional injection of living *Mycobacte-*

rium bovis. Science 172:271–273.

1309a. **Zeitz, S. J., J. H. Ostrow, and P. P. VanArsdel, Jr.** 1974. Humoral and cellular immunity in the anergic tuberculosis patient. A prospective study. J. Allerg. Clin. Immunol. 53:20–26.

1310. **Zeligman, I.** 1972. *Mycobacterium marinum* granuloma. A disease acquired in the tributaries of Chesapeake Bay. Arch. Dermatol. 106:26–31.

1310a. **Ziff, M.** 1974. Autoimmune processes in rheumatoid arthritis, p. 37–46. *In* L. Brent and J. Holborow (ed.), Progress in immunology II, vol. 5. Clinical aspects II. North-Holland Publishing Co., Amsterdam.

1311. **Zimmerman, L. E., L. Turner, and J. W. McTigue.** 1969. *Mycobacterium fortuitum* infection of the cornea. A report of two cases. Arch. Ophthalmol. 82:596–601.

1312. **Zobnina, V. P., and E. N. Astaf'eva.** 1970. Induction of pigment mutations in *Mycobacterium carotenum* by N-methyl-N'-nitro-N-nitrosoguanidine. Microbiology 39:894–897.